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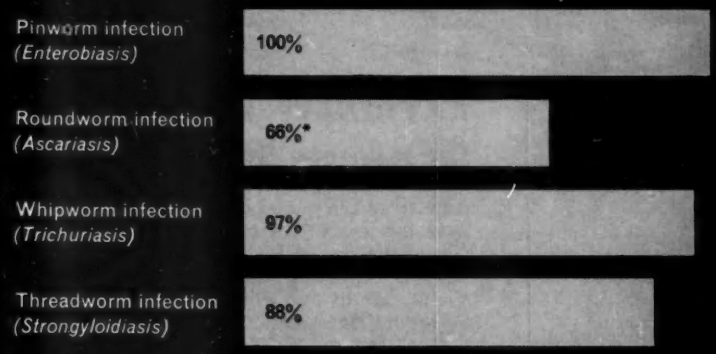

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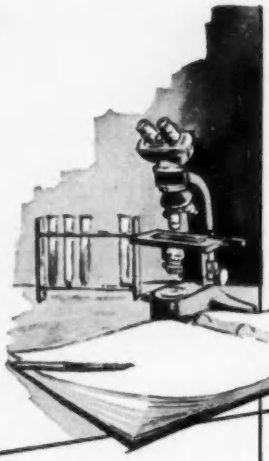

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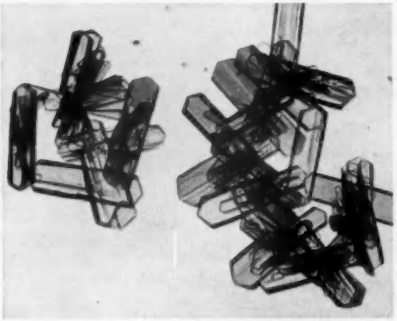

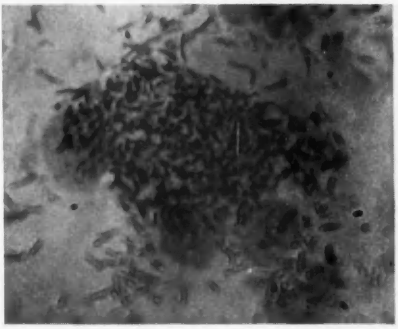

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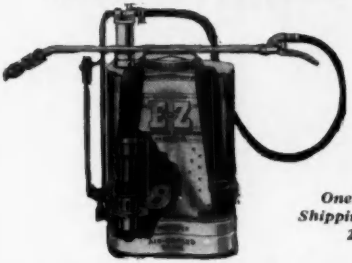

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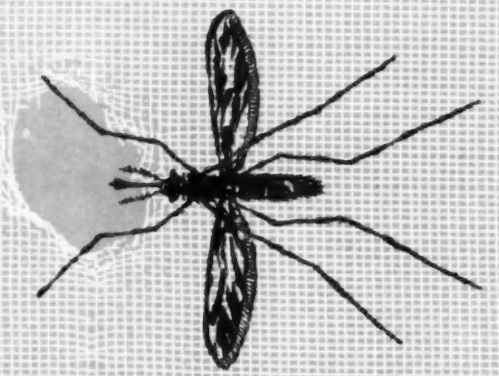

Asa Crawford Chandler

IN MEMORIAM

Asa Crawford Chandler (1891–1958)

Asa Crawford Chandler was born in Newark, New Jersey, February 19, 1891, of English-Danish parentage. He grew up in nearby Maplewood, always interested in animals, especially birds. After graduation from South Orange High School in 1906, he went to Cornell University, his choice because of its biology department. Receiving his A.B. in 1911, he obtained a graduate assistantship in the Department of Zoology at the University of California. This caused the Newark Evening News to print his picture with the legend "20-year-old youth made college professor;" long preserved as an item for Chandlerian chuckles.

Having followed Horace Greeley's advice to "Go West, Young Man, Go West," he never returned to the Atlantic seaboard to live. He received the M.Sc. degree in 1912 at Berkeley, and his Ph.D. degree under Professor C. A. Kofoid in 1914, with a 223-page thesis, reflecting his bird interests, on "A Study of the Structure of Feathers, with Reference to Their Taxonomic Significance."[1]

From 1914 to 1918 he was instructor, and assistant professor, in zoology and physiology, at Oregon Agricultural College, Corvallis. His first teaching assignment included a course in parasitology. Since there was no suitable American text at the time, he worked one up and became so interested in the field that it became his life's work. After a year of war service as 2nd Lieut., San. C., in 1919 he was appointed instructor in biology in The Rice Institute, recently established (1912) at Houston, Texas.

To the growing distinction of The Rice Institute he gradually added his own, as teacher (and beginning 1927, as Chairman of the Department of Biology), as resourceful investigator, as author, and as one of the American leaders of tropical medicine. His 39 years on the faculty there were interrupted twice by leaves of absence. During 1924–27 he was Officer-in-Charge, Hookworm Research Laboratory, School of Tropical Medicine and Hygiene, Calcutta, India. In 1952–53 a Fulbright award took him to the Fuad I Institute for Tropical Medicine, Cairo, Egypt.

Chandler's researches in parasitology initially rather centered on morphology and systematics, and to a lesser extent life cycles. These subjects he continued to investigate as opportunity afforded throughout his life, and they account for about half of his approximately 170 publications. He worked with all the groups of worms. Mainly brief and descriptive, his articles indicated unflagging interest in the manifestations of variety and degree of parasitism exhibited by both host and parasite species.

Active concern with the problem of man in relation to helminthiasis dates from the 3 years he spent in India. His studies there employed the newer methods for determining intensity of infection, as well as incidence. The results appeared mainly in 12 articles totalling 234 pages, titled "The Prevalence and Epidemiology of Hookworm and other Helminthic Infections in India." The size of the problem he had undertaken is evident in the subtitles of the series naming the places in which he surveyed: Eastern Bengal; Central, Western and Northern Bengal; Assam and the Hill Areas of Eastern Bengal; Tea Estates of Assam and Bengal; Burma; Bihar and Orissa; United Provinces of Agra and Oudh; North-West India (Punjab, Kashmir, Rajputana, North-West Frontier Province, Baluchistan and Sind); Central India and Bombay (Central Provinces, Central India Agency and Gwalior, Bombay Presidency except Hyderabad); Madras Presidency and Southern States. The final summary article[2] remains a permanent contribution to the interpretation of the then-existing intestinal helminthiases of man in the Burma-India-Pakistan region.

Treatment campaigns were not involved in his work. However, supplementary to the epidemiological investigations, he engaged in studies on the efficacy of vermicides with Major R. N. Chopra of the Indian Medical Service. This fruitful association resulted in a volume on "Anthelmintics and Their Uses in Medical and Veterinary Practice" that is still one of the few treatises in this so slowly-growing field.[3]

The experience in India also activated Chandler to bring to focus at book length in 1929, the

subject of worldwide hookworm.[4] There had been no comprehensive summary of the problem later than that of Dock and Bass in 1910, and none has been added since except the largely supplementary volume by Clayton Lane in 1932.

The period when Chandler wrote "Hookworm Disease" was after the stirring decade of field and laboratory researches on hookworm in various parts of the world that had literally reoriented our knowledge of this infection in man. His presentation of the distribution of hookworm, its biology, epidemiology, pathology, diagnosis, treatment, and control, became a valued epitome of the new insight and perspective that had been gained in relation to this worst of man's helminthic pathogens. As a major record of the hookworm problem it should, with time, play a role in the renaissance of interest that must lie somewhere in the not-too-distant future when effective hookworm control on a worldwide basis will be undertaken anew.

Following his tropical experience, and back at The Rice Institute, Chandler turned attention to the newly developing experimental problem of susceptibility and resistance to helminthic infections. In 1932 he furnished an early review[5] of the field as it then appeared, and included some results from the first of his own experiments with *Nippostrongylus muris*. As he pursued the latter he came to conclude from the experimental results, as other investigators were increasingly doing with various worm-host systems, that nutritional factors of neither parasite nor host could be neglected. In a broad way this had long been orthodoxy to observers of parasitism. It was one of Chandler's contributions that it led him and his students to more detailed inquiries into the physiology of helminthic parasitism and host resistance.

This whole experimental area gained significantly in impetus and understanding through the persistence and ingenuity of Chandler and his group in analyzing the host-parasite interplay, first with nematodes, later with cestodes and acanthocephala, and more recently with *Trypanosoma* and *Trichomonas*. His own developing perspective is revealed in certain of his review articles,[6] the most recent being the summary of his lectures at the Kasr-el-Aini Faculty of Medicine, Cairo, 1953, on "Immunity in Parasitic Diseases."[7]

Increasingly, the nutritional aspects of host-parasite relations led Chandler to greater preoccupation with their implications. Somewhat differently than in his popular "Eater's Digest,"[8] he made "The Relation of Nutrition to Parasitism" the subject of another series of lectures in Cairo.[9] Later, "Interrelations between Nutrition and Infectious Disease in the Tropics" was the title of his retiring address as president of the American Society of Tropical Medicine and Hygiene,[10] and "The Role of Malnutrition in Helminthic Infections" was the subject of the report he submitted for presentation at the Lisbon meetings.[11]

The central interest and accomplishment of Chandler and his laboratory developed a memorable event in January, 1958. A new biology building at The Rice Institute was then dedicated with a notable 3-day symposium on "Resistance and Immunity in Parasitic Infections."[12] This was attended by parasitologists, including most of his former graduate students, from all parts of the United States. They came to Houston not only because of the character of the program, but because they were proud of Asa Chandler and wished to honor him. To repeat part of my own foreword on the occasion, "the parasitological fraternity of the whole world—if I may make bold to speak for it—shares that pride and affection."

Chandler, in his Introductory Remarks at this symposium, outlined the development of biology and parasitological thinking at The Rice Institute, and something of the researches of himself and students. Of these the first, Paul D. Harwood, Ph.D., 1931, was chairman of one of the sessions, and another, Clark P. Read, Ph.D., 1950, gave a symposial report. In another place[13] the latter has expressed a bit of the formula of Chandler's relation to his students. The range of requirements for successful endeavor in a complex field Chandler had himself explored in a presidential address (1946) on "The Making of a Parasitologist."[14] It is worth noting that sidelights of this problem had earlier been felt to deserve discussion from the same rostrum by presidents M. C. Hall (1932) and E. E. Tyzzer (1934) and, later, by E. C. Faust (1948) and C. G. Huff (1955).

Other of Chandler's studies over the years attest the ramifications of his interests. In 1920 he wrote of the "Control of Fluke Diseases by

Destruction of the Intermediate Host."[15] Basing his recommendation on experiments, he became the first to advocate use of copper sulphate for killing aquatic snail vectors in their natural habitat. This chemical was for years the prime molluscicide, and prodigious research in this field, especially since World War II, has not yet quite displaced it.

In 1923, Chandler and Lee Rice[16] gave an account of an epidemic of dengue of unprecedented proportions which occurred in 1922. This accompanied a scourge of *Aedes aegypti* such as had not earlier been recorded in the Southwest. The number of cases in Texas was later estimated by the State Board of Health as between 500,000 and 600,000. The Chandler-Rice observations, including successful transmission experiments, led them to agree with other investigators who believed that the reputed relationship of dengue and yellow fever had been overemphasized, and that it was improbable dengue was caused by a leptospira, an idea then having some currency. Their findings favored the demonstration, in 1907, by Ashburn and Craig of a filtrable virus as the etiological agent.

In 1941, with Melba Lee, he published[17] on a worrisome Texas problem, namely on the nature, growth, and, as a result of their study, recommendation for control of bacteria in oil-water emulsions used as lubricants in the cutting of metals.

In 1945, reporting as a Special Consultant, Malaria Control in War Areas, United States Public Health Service, he clarified some of the factors influencing the uneven distribution of *Aedes aegypti* in Texas cities.[18]

The diversity of Chandler's biological interests that centered in parasitology was nourished by an activity that spanned nearly 4 decades, namely the writing, revising, and rewriting, through nine editions (1918–1955) of his textbook.[19] It came to be so useful a contribution to the elucidation of its field in his generation that he in turn came to be, in a way, Professor of Parasitology to the world. The "Introduction to Parasitology" was "appreciated by students and experts alike," as expressed by H. W. Brown in 1955. To have reached a circulation of 75,000 copies over the years, and in its last two editions to have been adopted for courses in 420 colleges and universities, including a dozen outside the United States, indicated unusual qualities.

I have sought from some of his reviewers since the first edition why "the book is excellent" (A. Alcock, 1918). Three aspects seem especially emphasized. First, "the work impresses one as a unit rather than a patchwork" (H. B. Ward, 1922), "better than any other it relates the general biological story of animal parasitism" (J. T. Self, 1955), "a fascinating integrated study of living organisms" (W. E. Kershaw, 1957). Second, and most frequently mentioned, "the clarity of expression,* the pleasant readability" (D. H. Wenrich, 1955), "one of the most readable and entertaining books upon the subject in English" (C. F. Craig, 1936), "written lucidly and with a characteristic vitality and humour" (H. I. Heinz, 1956), "the gift of the graphic and imaginative phrase; he can put, often in a simple arresting sentence or two, the complex relationships of the parasite and its environment" (G. La Page, 1956). Third, its generally admirable perspective, the "global aspects kept constantly in view" (M. E. Barnes, 1950), and, significantly, "there is little evidence of scientific nationalism in the book" (K. Mellanby, 1956). If these be marks of his competence, they may also be regarded as measures of a standard.

He was in 1958 in process of rewriting the 10th edition, to be undertaken with the joint authorship of Clark P. Read, who is to complete the revision.

To those interested in parasitology from the biological side, as well as from the side of tropical medicine, Chandler put The Rice Institute in the category of Emerson's better mousetrap. Among his professional activities especially to be mentioned were such responsibilities as being the Section Editor on Parasitology of *Biological Abstracts* for 20 years, member of the Editorial Board of the *Journal of Parasitology* for ten, and for *Experimental Parasitology* since its establishment. He served two 5-year terms on the Tropical Medicine and Parasitology Study Section of the National Institutes of Health for the awarding of research grants, and was on the Expert Advisory Panel on Parasitic Diseases of the World Health Organization. He was a former president

* In April 1958, a letter to him had mentioned my envy of "people like you who achieve clarity by first intention, so to speak," and he had replied, "Thanks for your remarks about the achievement of 'clarity by first intention'—it is entirely undeserved, for I write, rewrite, and rewrite."—N.R.S.

of the American Society of Parasitologists (1945), the American Microscopical Society (1950), and the American Society of Tropical Medicine and Hygiene (1956). For the Sixth International Congresses on Tropical Medicine and Malaria meeting in quinquennial session in Lisbon in September, he was accorded the distinctive honor of being named as one of the five Honorary Presidents of the Congresses, the only one from the United States.

He died in Rotterdam August 23, 1958, while enroute to Portugal.

NORMAN R. STOLL,
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THE WORM TURNED—ACTION AND REACTION*

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My reason for selecting a subject on worms for this address is that my professional career has been largely devoted to the study of parasitic worms, and I have always found these creatures interesting and at times exciting. It is, therefore, another instance of "Doing What Comes Naturally."

At what time man first attached any significance to the action of worms must be a matter of pure conjecture; it is quite unimportant. But at one time, somewhere along our ancestral trail, someone possessing that rare quality, curiosity, stopped long enough to watch a crawling creature; he probably poked at it, and also, probably to his astonishment, noted that the creature turned sharply against the poker. The observation provoked an idea: the proverb, "*The worm turned.*" Thus, a saying burst forth. It was pleasing, it spread, became commonplace and, let us say with all due credit to Theodor Storm, the author of that delightful novelette, "Immensee," it sprouted, it dropped from a cloud, floated over land like gossamer and was sung in a thousand places at the same time. It became a favorite epigram of poets and scribes.

You may well recall that Clifford, in Shakespeare's "Henry IV," cautions his gracious liege that, "The smallest worm will turn, being trodden on." And, Cervantes, being more than a little vexed over the publication of a second "Don Quixote" while he was preparing his own second part for press, notes in his foreword, "Bless me, Reader, gentle or simple, or whatever you may be, how impatiently by this time must you expect this Preface, supposing it to be nothing but revengeful Invectives against the Author of the second Don Quixote. But I must beg your pardon—though it be universally said, that even a Worm when trod upon, will turn again, yet I'm resolv'd for once to cross the Proverb." And again, Thomas Randolph in his entertaining "The Muse's Looking Glass" takes cognizance of the fact that,

> "Poor worms being trampled on turn tayle,
> As bidding battail to the feet of their opressors."

This observation was made in 1638. I do not hold that it is exactly the precursor to the discovery of dermal infection made 260 years later by Professor Looss.

Despite its great age, the phrase has never grown stale or hackneyed. I would remind you of its fresh appearance in Roger Lee's fascinating autobiography, "The Happy Life of a Doctor" which was published just two years ago.

"The worm turned" was very much of an old saw to me until one day it suddenly came to mind and it carried new significance; in fact, it had a very singular meaning. It occurred some time ago during one of our symposia on malaria. The speaker, who was reviewing the history of malaria, at the moment was dwelling on the origin of Manson's mosquito-malaria theory. The old saw burst upon me, for it was indeed the *turning of a worm* that prompted Manson's theory; it ushered in a new era in medicine, it was the beginning of modern tropical medicine. Reflecting for the moment, I ceased to follow the speaker. I sat there recalling some of the turnings of worms which had had direct and forceful influence on the development of tropical medicine and tropical public health. I assure you I had visions of actions and reactions.

Now, seemingly going somewhat astray, I am going to dwell briefly on a certain phase of physics, a phase of the subject which was of particular interest to all of us early in our scientific careers; in fact, much before we decided upon a scientific career, namely, Newton's third law of motion; "To every action there is an equal and opposite reaction." Objects which turn are objects in action. Although the action and the reaction are always equal, the action may be readily perceived but the reaction may not be noticeable. For example, when a person pushes his stalled automobile along the street, the force

* Presidential address before the American Society of Tropical Medicine and Hygiene at its annual meeting, November 7th, 1958 at Miami Beach, Florida.

he applies against the automobile is equal to that applied against the pavement. The action is perceptible, but the reaction is not. Let the pusher put on roller skates and the reaction becomes immediately and decidedly apparent. In other words, the roller skates were added equipment which made visual perception of the reaction possible. Similarly, the reaction to the turning of a worm was not sensed until comparable *added equipment* was applied. In the present presentation, reaction will refer to man's understanding and appreciation of the worm's action, rather than to a direct reaction; namely, any pathologic physiology.

In all probability our parasitic worms in ancient times did as much turning as they do today; there was the same action on the part of the worm but the equipment for sensing any reaction was lacking. Essentially there was little or no reaction. It is true that the commoner, cosmopolitan helminths, *Ascaris lumbricoides*, *Enterobius vermicularis*, and the larger tapeworms were known. Primitive man as well as the medievalite must have been well aware of these creatures. They did not know how they got into the body, but they were fully cognizant of the way they left the body. To some, they were the obvious cause of their intestinal disorders, abdominal pain, while to others their presence was an indication of normal health and well being.

However, once it was assumed that these worms could cause pain, it was natural first to suspect, and later conclude, that worms caused pain and illness elsewhere in the body, particularly the most violent pain, the toothache. The symptom was the disease, as a rule, but in other cases the cause of the symptom was considered the disease. The patient had worms, the worms were the disease. Thus, action was observed, but the reaction was overlooked except by the physicians who prescribed remedies against the worms or the disease. The naturalist, the zoologist of the day, speculated on the question of the origin of worms. Further than this point, it mattered little if the worm turned, for to them, it looked much the same on both sides. This trend of thought remained unchanged until well into the 17th century.

Then came an abrupt and glorious awakening, a time of spiritual and intellectual uplift, an age of *individual* scientific endeavor and unbiased observations. As far as the worms were concerned, that bit of added equipment permitting accurate observation of action and reaction was introduced, namely Leeuwenhoek's simple lens of small size and with considerable curvature for close focus. The study of intestinal worms became the vocation and avocation of the naturalist. It became a specialty; the reaction became more apparent than the action and a new branch of zoology, helminthology, came into existence.

The present long list of helminthologists could rightly begin with Marcus Bloch, Johann August Goeze, and Peter Simon Pallas, whose years were from 1723 to 1811. Whether these gentlemen would have accepted such distinction is, I believe, a bit of a question for each is well known, if not better known, for contributions in other fields; two were physicians and the third was a theologian. But all had a common avocation; they were naturalists in the true sense of the word and probably would have preferred to remain in this category.

Even in the present day of specialization it is difficult, if not hazardous, to place a scientist in any definite category. He might disagree with your classification. For example, some years ago at an evening seminar the then recent studies on bird malaria were to be presented. The speaker was the late Dr. Samuel T. Darling. The chairman reviewed Dr. Darling's outstanding scientific contributions and introduced him as a protozoologist. I was looking at Dr. Darling and noticed that he shuddered a bit; then he rose to speak, beginning somewhat as follows, "Gentlemen, I have been introduced to you as a protozoologist. Urumph! This is indeed something new and strange to me. I always considered myself a pathologist—and I hope I shall be remembered as such."

During the 18th and 19th centuries helminthology was in its heyday, and there were helminthologists of that day: Küchenmeister, Herbst, Colbald, Steenstrup, van Beneden, and many others; and especially Leuckart, affectionately recognized at home as *Altmeister der Helminthologie* and acknowledged as the great leader by students in all nations. The apparent force of the reaction equalled that of the action. Thousands of animals were examined for their worm parasites and records were made. It was discovered that a worm does not look the same on both sides. Sex was revealed. The experimental method was successfully introduced

into research, life histories were discovered and the widespread belief in the myths of necrogenesis and abigenesis became lost in oblivion.

I look upon the elucidation of the life-history of *Trichinella spiralis* as the first epochal contribution of helminthology to the medical sciences, particularly to the fields of epidemiology and public health. You will recall that this worm was first described in 1835. Twenty-five years later it was demonstrated that *T. spiralis* could cause fatal illness in man and that, almost immediately following this discovery, the disease in Germany was recognized in epidemic proportion with high mortality. In the meantime the parasite had been of keen interest to the helminthologists. Herbst demonstrated the transfer of the worm from one animal to another. Leuckart, Virchow, Küchenmeister and others, by careful feeding experiments with laboratory animals, had worked out its complete life cycle, and Leidy had discovered trichinae in the flesh of swine. Studies of the great epidemics in Germany clearly showed that the disease was of porcine origin and government action for control was applied.

Epidemiology, due to the early influence of Sydenham, reached a high state of development during the 18th and 19th centuries, in the hands of British epidemiologists. The epidemiological inquiries of the time were, however, mainly directed toward establishing the distribution of the epidemic in time and space, the historical and inductive methods of epidemiological research. The cause was unknown and the methods for control lacked scientific support. The investigation of the epidemic was often not begun until after its termination. Thus, the early studies on *Trichinella spiralis* ushered in a new method of epidemiological research, the *parasitological* method, for here is the first epidemic disease for which both cause and transmission were fully known and for which sound control measures were available at the time of the epidemic.

Likewise, the first endemic disease of which the agent, host, and environment were known was of helminth origin, namely echinococcosis. This knowledge was gained through the application of the parasitological method of research and it led to astonishingly effective means of control. Prophylactic measures against the disease were proposed in 1863 to the Icelandic Ministry of Health and these measures were rapidly put into action. Prior to this time the prevalence of echinococcosis in Iceland was astonishingly high. Following the application of control measures there was a remarkable and steady decline in the number of cases. Sanborn visited Iceland in the early 1920's. Later, in an address before the Institut Pasteur, Paris, Sanborn remarked, "Iceland has long been known as the classic land of hydatid disease, but nowadays echinococcosis has diminished in frequency as strikingly as yellow fever at Panama following the application of preventive measures based on true and precise etiological knowledge." Today there appears to be no echinococcosis in Iceland. These are examples, par excellence, of reactions to the "turning of a worm."

When Dubini, in 1838, discovered hookworms in the intestines of an Italian peasant, his reaction appears to have been scarcely more than a fleeting glance. Fifty years later hookworms were recognized as the cause of a violent epidemic of anemia, that of Saint Gotthard, and twenty-five years after this epidemic, hookworm disease was recognized as a major public health problem on every continent. Hookworm control was a potent force in the introduction, development and extension of public health services throughout the world, particularly influencing their development in rural areas.

From the very beginning, public health services were designed primarily for the improvement of the health and comfort of the city dweller and to protect the city from epidemic disease. Even the later developments following the advent of bacteriology, food sanitation, methods for tracing epidemics, laboratory diagnosis, the preparation of vaccines and vaccine prophylaxis, were to a large extent developments of bacteriological laboratories within the city and for urban populations. The countryside, from ancient times, was considered a healthy environment and a refuge in times of pestilence.

Very late in the year 1899 Bailey K. Ashford saw a worm turn; in fact he saw thousands of these particular worms turn. All were parasites recovered from rural Puerto Rican patients; the peasants, the *jibaro*, sick and so utterly miserable that, according to Ashford, they could not be more so. Ashford proved the parasites were hookworms and a cause of the malady which was rampant throughout the rural areas of the island.

Ashford's reaction was explosive; it was

epochal. Following the formal announcement of his discovery, Ashford made a strong appeal that government action be taken to combat the existent conditions. I will not relate the steps in the development and extension of hookworm control to rural areas throughout the world. These are well known by all present. I will note only that wider health services to rural communities followed the hookworm campaign as their benefits were realized. It can be rightly said that the foundation of rural public health was laid when it was realized that hookworm disease was a reality and that it constituted a serious menace to rural health and prosperity. This discovery marked the awakening of health consciousness of rural areas; a definite reaction to the "Turning of a worm."

There were many 'side reactions', or should I say 'chain reactions' to the turning of the hookworm. Of these, I shall briefly mention only two which I hold to be of particular interest and significance. These were the development of anthelmintic medication and the introduction of parasitology into the curricula of our medical schools.

Late in the 19th century, when ancylostomiasis was recognized as the cause of the Saint Gotthard epidemic, the known vermifuges proved to be worthless. An eager and competitive search began immediately for an effective drug. Bozzolo, in 1879, found thymol to be highly effective. Its value was confirmed by others, and it became almost a universal remedy and the drug of choice for nearly thirty five years. The search for better anthelmintics was, and is, continuous. In 1921, Hall discovered carbon tetrachloride to be remarkably effective against hookworm infection and, in 1925, he announced the discovery of tetrachlorethylene as a new anthelmintic, believed to be safer and even more effective than carbon tetrachloride. It remains today as the drug of choice against hookworm infection.

It was the results of crude experiments in which hookworm larvae were subjected to various chemicals which led to the use of thymol as an anthelmintic. However, its value as an anthelmintic, as well as the value of other anthelmintics, was judged for many centuries mostly by clinical improvement and the passage of worms. Our knowledge of the value of carbon tetrachloride and tetrachlorethylene as anthelmintics came from carefully-planned studies with critical testing on experimental animals. Such critical testing of anthelmintics was first approximated in 1884 by Grassi and Calondruccio. The method was overlooked, or at least unused, until it was reintroduced in 1902 by Stiles, and later developed in the United States Bureau of Animal Industry under Ransom. Hall did not limit his studies on anthelmintics to animal experimentation. To gain information on the safety and subjective effects of tetrachlorethylene, Hall ingested the drug one day shortly after breakfast, which included liver and bacon, both probably contraindicated, drank milk later which is also contraindicated and indulged in smoking throughout the day. As I recall, Dr. Hall was a fairly heavy smoker. He reported that the only apparent effect of this self-medication was a complete relaxation of the muscles on retiring that night leading to an unusual dream of levitation. It seems very likely that Hall's report never reached the masses; no addiction to tetrachlorethylene has been noted among the thousands who have tasted this little-known euphoriant.

Prior to the turn of the present century, parasitology was primarily a field for academic graduate study. It seems to have been given very little, if any, attention in the medical schools. In 1891, Charles Wardell Stiles returned to the United States after a period of study in Europe, to enter government service in the U. S. Bureau of Animal Industry. He had received the Ph.D. degree from the University of Leipzig where he studied under the renowned and immortal helminthologist, Rudolf Leuckart. The following year Stiles was lecturing in parasitology before the medical students of Georgetown University, the Army Medical School and Johns Hopkins University. He later introduced the subject in Medical Schools of North Carolina, Georgia, and Louisiana. Hookworm bionomics and hookworm disease must have remained foremost with him from his Leipzig days, for in his lectures to these students he emphatically stressed the possible existence of hookworm disease in the United States and he cautioned his students that, if they found cases of anemia in man in the tropics or subtropics, the cause of which was not clear, they consider the possibility of hookworm disease, make a microscopic examination of the feces and look for eggs of the parasite. It is of interest to note that Bailey K. Ashford had attended Stiles' lectures both in the George-

town Medical School and the Army Medical School.

It cannot be said, however, that ancylostomiasis was of primary, or even secondary, influence in bringing parasitology into the curricula of all of our medical schools. Certainly it was not at the Harvard Medical School. In this school such recognition was a sequel of Theobald Smith's course, "Comparative Etiology of Infectious Diseases," first offered during the second half year in 1898. The content of this course is not described in the school catalogue of that year, but the catalogue of the following year states that the course was a fourth-year elective, and limited to men qualified to do original research work. It consisted of lectures and demonstrations on the comparative etiology of infectious diseases, general principles underlying infection, and on public health problems arising from infectious diseases of animals.

In 1900 three optional courses were added to pathology, one of which was a course on the pathology of certain parasitic diseases, given by Theobald Smith. Parasitic worm diseases probably were included, but none is mentioned. In 1901 the content of this course is more clearly defined: it was a course of lectures and laboratory exercises on animal parasites, particularly protozoa, and the infections produced by them. The annual examination in pathology for the following year included for the first time a question on parasitology, namely, "Give the complete life-cycle of the blood-parasite of tropical malarial fever as now generally accepted." Some questions on parasitism in the annual examinations in pathology for the next few years were comprehensive and would require answers mentioning hookworms. But no direct, specific, question on ancylostomiasis appears until 1907 when the student was asked to give the biology and pathological action of *Uncinaria*. Therefore, it seems quite clear that the introduction of parasitology at the Harvard Medical School was not a reaction to a worm's action, but rather to that of a protozoan which was, I suspect, *Babesia bigemina* Smith and Kilborne, 1893.

From the foregoing, it would appear that the development of helminthology was a reaction to worms affecting human beings and animals. This is the impression all of us could have gained from our college courses in biology and very likely also from graduate studies in parasitology. This is a very one-sided impression and it is due to the fact that, although our texts on biology and parasitology expressly deal with the important parasites, the word 'important' invariably means important to *man* and *domesticated animals* and, particularly, 'important' to *man*. The helminths parasitizing plants are either ignored or are given the slightest attention. Yet these plant-inhabiting helminths are world-wide in distribution, they rank among the worst agricultural pests and their significance as limiting factors in crop production is unknown.

I should like to give a specific example: Thirty years ago on the Island of Bangka which lies off the east coast of Sumatra, hundreds of acres were devoted to the growing of black pepper. The economy of this island was based on this crop. Today, due to a particular burrowing helminth, the pepper industry on this island has been wiped out. This same worm parasitizes citrus; it has already affected 8,000 acres of citrus in this, the State of Florida.

The plant-inhabiting helminths are all nematodes and include both obligatory and facultative parasites. Despite the fact that the first species was discovered more than a hundred years before the discovery of *Trichinella spiralis* was announced, and that its life-history stages and pathogenicity, *i.e.*, gall formation, were known almost a hundred years before trichiniasis was recognized as a disease, the plant nematodes interested but few of the early helminthologists and remained neglected until fairly recent times. Among the pioneers I would mention Needham of England, Kühn and Steinbuck of Germany and Dovaine and Chatin of France.

In 1907 a most remarkable man joined the United States Department of Agriculture as 'Agriculture Technologist'. He was enthusiastic, visionary, mathematical, and possessed of keen and rare sense of humor. It is rightly said of this man that he embodied all that is meant by a "scientist." He was Nathan Augustus Cobb; Nay Cobb to his close associates. Cobb's early fields of interest were in standardization of cotton types and free-living and plant nematodes. Under him the study of plant nematodes became a distinct and separate branch of helminthology, the study of nemas, or nematology; names introduced by Cobb, from which there were numerous derivations: "nematologist", "nematize", "nematosis", "nematicide." Cobb became 'Senior Nematologist' in 1924 and 'Princi-

pal Nematologist' in 1928. The category 'Nematology' was expressly created for Nathan Cobb, its meaning being restricted to studies on plant-insect-inhabiting and free-living nematodes.

Nematology, unfortunately, is still a much underdeveloped science, but the reaction to nemas is constantly gaining force. In 1950 the only agencies in the United States dealing exclusively with nemas were the Division of Nematology in the United States Department of Agriculture and the then-newly-established unit at the College of Agriculture and the Agriculture Experiment Station of the University of California. Today, less than 9 years later, there have appeared departments, sections, or currently-given courses presided over by trained phytonematologists (an even newer category) in the universities or land grant colleges of Alabama, California, Florida, Maryland, New York, North Carolina and Wisconsin.

I have to a large extent drawn attention to the actions and reactions of worms of earlier years. I should now like to consider briefly the nature of these forces today; first the magnitude and then the variability; whether the forces are static, and if not static, what is the direction or, in other words, what is the trend.

To appraise the magnitude, let us examine the record, namely, "Helminthological Abstracts." This exceptionally valuable source of information on all the many and varied applications of helminthology began publishing in 1932. In that year Volume I contained abstracts from 268 periodicals; 776 authors had published 873 papers. Volume V contained abstracts from 458 periodicals; 1,199 authors published 1,346 papers. Except for the war years there were steady expansion, more periodicals, more authors, more publications. Volume XX contains abstracts from 696 periodicals; 2,113 authors published 2,067 contributions.

I shall now point out the course helminthology has taken as it is reflected in the publications in our own Journals. First, let us turn to Volume XII, 1932, of the American Journal of Tropical Medicine; this is, as you may recall, the year Helminthological Abstracts made its appearance. A total of 39 papers were published, three of which were on helminthiases, namely, one on skin hypersensitiveness to hookworm antigen, one on complications of filariasis and elephantiasis and one on the treatment of intestinal parasitism by transduodenal irrigation with hot physiological salt solution. This reflects the pattern of the Journal, as far as publication in the field of helminthology is concerned, from Volume I, 1921, to Volume XXIII, 1943. Papers on helminthological subjects made up approximately 10 per cent of our total scientific contributions.

A change, a concentration on certain phases of helminthology, begins with Volume XXIV, 1944. In this volume only six of the 57 contributions deal with helminths, but five of these papers have to do with filariasis; and two of these announce filariasis in American troops serving in the South Pacific area. Volume XXV, 1945, announces hyperendemicity of schistosomiasis on Leyte Island, another theater of the war. Eighteen, or more than 22 per cent, of the contributions deal with helminthiases; of these, 12 have to do with filariasis and five with schistosomiasis. Schistosomiasis and filariasis continue to be foremost subjects in The American Journal of Tropical Medicine and Hygiene. Volume V of our new Journal published a total of 125 contributions of which 44, or 35 per cent, deal with helminth infections. Twenty-two of these papers are on schistosomiasis and four are on filariasis. The same pattern obtains today. Number five of Volume VII, which has just appeared, contains fewer original contributions, 17, but three of these deal with schistosomiasis, two with filariasis and three are concerned with insect vectors of filariasis. The emphasis remains on host-parasite relationships, epidemiology and control.

World War II is responsible for this trend; concentration and markedly increasing activity concerning two tropical diseases which heretofore, in this country, were matters of special interest to only a relatively few research workers, physicians connected with medical missions or with industrial firms operating in the tropics. World War II ushered in a sudden change. It was somewhat of a rude awakening to the fact that the whole world had shrunk, that our public health problems were no longer limited to continental U.S.A., and that the solution of the health problems of the warm climates, particularly health problems of parasitic origin, are of primary importance. From the outset the reaction was international. Never has there been greater impetus to mobilize the resources of

the Free World to combat and abate disease and to improve living conditions, not just in the Tropics, but in all parts of the world. The aim has been towards international friendship and peace, international cooperation "For survival instead of destruction." Surely, the worm turned; it keeps on turning; there is action and there is reaction. The reaction is clearly visible and distinct; it is definitely equal and opposite to the action.

In closing, I want to express my deep appreciation to those who gave me the privilege of serving as President of our Society. For me it has been an honor, a very singular honor and pleasure.

PRESENTATION OF THE WALTER REED MEDAL FOR 1958

TO

DR. WILLARD H. WRIGHT*

WILLIAM A. SODEMAN

Chairman, Walter Reed Medal Committee

Mr. President and members of the American Society of Tropical Medicine and Hygiene:

The Walter Reed Medal Committee, composed of Doctor Andrews, Doctor Taliaferro, and myself, is pleased to nominate Doctor Willard H. Wright for the 1958 award. The nomination was unanimously approved by the Council of the Society; and I, as Chairman, have the honor to present the medal to Doctor Wright.

The first Walter Reed Medal was conferred in 1936. Recipients in the past include such names as William B. Castle, Herbert C. Clark, Carlos J. Finlay, Paul F. Russell, James Stevens Simmons, N. H. Swellengrebel, and Rolla E. Dyer. The Committee is most pleased to add Doctor Wright's name to this impressive list.

Willard H. Wright was born in Findlay, Ohio. He received the degree of Doctor of Veterinary Medicine at George Washington University in 1917 and began his career with the United States Department of Agriculture in 1919 as a practicing veterinarian. He served in various field assignments until 1928 when he turned his attention to the problems in parasitology. In 1935, he received the degree of Doctor of Philosophy in Zoology from the George Washington University. His thesis included one of the first studies on the relationship of vitamin deficiencies to parasitic infections. In 1936, he joined the staff of the National Institutes of Health as a senior zoologist. By 1939, he had become Chief of the Zoology Laboratory and, since 1947, has served as the Chief of the Laboratory of Tropical Diseases. In 1948, he accepted the added responsibility of Assistant Director, National Microbiological Institute, National Institutes of Health, and continued in this capacity until 1955.

In his first studies on public health problems in parasitology, he made significant contributions in the development of the gentian violet treatment for oxyuriasis and in working out the detailed epidemiology of trichinosis in the United States. His papers on these subjects are classics of good scientific thought and expression. He recognized very early the importance of schistosomiasis as a widespread disease of economic moment and of military importance. He provided much of the stimulation for the work on onchocerciasis in Guatemala which led to an understanding of the epidemiology of the infection, the biology of the vectors, and the proper use of certain chemotherapeutic agents. He was responsible for initiating work on toxoplasmosis. In his capacity as Professorial Lecturer at George Washington University, he has guided a number of students through doctoral work in parasitology in which most of the work was oriented toward basic problems. In more recent years, he has developed a broad appreciation of problems in germ-free animal research of parasitic diseases and has been instrumental in setting up work in this field at the National Institutes of Health.

In recognition of these outstanding abilities, he has received many honors. He has been President of the American Society of Parasitology and Vice President of our Society. He has served on numerous international organizations, commissions, and agencies.

The American Society of Tropical Medicine and Hygiene, therefore, takes pleasure in presenting the Walter Reed Medal of 1958 to Dr. Willard H. Wright in acknowledgment of his contributions to the fields of parasitology and protozoology, of his contributions in the application of such knowledge to public health practices, prevention and therapy, and in recognition of his stimulus and guidance to others in their development in this field. Through his students, his work is being carried forward.

* At the annual meeting of The American Society of Tropical Medicine and Hygiene, Miami Beach, Florida, November 7th, 1958.

AN APPRAISAL OF THE ENTEROVIRUS PROBLEM*, †

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I am deeply appreciative of the privilege of presenting the 23rd Charles Franklin Craig Lecture. As a virologist, I learned early of Dr. Craig and of his achievements as investigator and codiscoverer of dengue virus, as expert in tropical medicine, as educator and as editor for 20 years of our Journal. During the active period of Dr. Craig's lifetime, only 21 viral diseases of man were recognized. In the past 10 years, understanding of viral diseases has grown prodigiously. In this single decade, more than 100 hitherto unknown viruses have been characterized and many others under study are known to be distinct from established types.[25, 55] Of these hitherto unknown viruses, about 50 are in the human enterovirus tribe made up of Coxsackie, ECHO and polioviruses[9] (Table 1). The relationships of viruses within the tribe will be considered later. Rapid accumulation of evidence from laboratories in all parts of the world has made it known that these viruses constitute a continuing threat to residents in tropical, temperate and arctic zones. Indeed, viral diseases are replacing protozoal and bacterial infections as the dread diseases of the tropics. Time today permits only an appraisal of the enterovirus problem. I shall begin by referring briefly to the three key contributions that opened the way for laboratory approach to a vast number of disease problems.

The first contribution concerns poliomyelitis and the polioviruses. In our present state of sophistication, it seems a long time back to 1948 when unequivocal evidence had not yet been established for the existence of more than one immunotype of poliovirus. Indirect evidence gathered by many laboratories over the years had made it apparent that more than one immunogenic type of poliovirus was responsible for the human disease. But how many types of virus and how they differed were unknown. This problem was resolved conclusively in 1951 when the Committee on Typing of The National Foundation for Infantile Paralysis[10] published evidence that three distinct immunogenic types of poliovirus are responsible for clinical poliomyelitis. This evidence came from a cooperative study begun in 1948 by four teams of investigators located at the Universities of Pittsburgh, Kansas, Utah and Southern California. Three years later, from use of countless monkeys and expenditure of about $1,300,000, two hundred strains of virus from patients all over the world had been allocated into three distinct immunotypes, poliovirus types 1, 2 and 3.

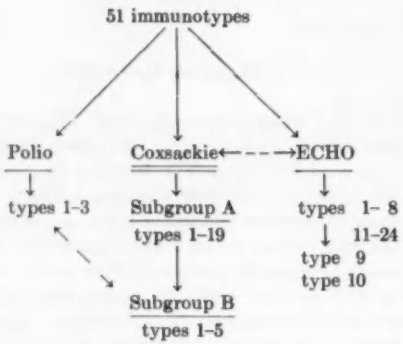
The second key contribution was the introduction of the suckling mouse as a tool for isolation and study of enteroviruses. Use of newborn mice in 1948 by Dalldorf and Sickles[12] for virus assay of feces of two children suffering from poliomyelitis in the little town of Coxsackie in New York State resulted in recovery of a virus pathogenic for newborn mice but nonpathogenic for older animals. Successive observations by Dalldorf and his associates and others[11] led to routine use of suckling mice for virus isolation, and to recognition of two virus subgroups separable by antigenic differences and characteristically distinctive pathologic response: Group A Coxsackie viruses induce flaccid paralysis, myositis and muscle degeneration; Group B Coxsackie viruses cause encephalomyelitis, pancreatitis, focal myositis and/or fat necrosis. The suckling mouse continues to be the medium of choice for isolation and study of Coxsackie virus types A-1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 17 and 19. Either suckling mice or cell cultures can be employed alternatively for isolation and study of Coxsackie B-1, 2, 3, 4, 5 and A-9. Stable cell lines of human origin can be employed for study of types A-11, 13, 15 and 18. In our laboratory cultures from primary human amnion are now in use for the study of Coxsackie virus types A-6, 9, 11, 13, 14, 15, 18 and types B-1, 2, 3, 4, 5.

* The Twenty-third Annual Charles Franklin Craig Lecture delivered before The American Society of Tropical Medicine and Hygiene, November 5, 1958.

† Aided by a Grant from The National Foundation.

TABLE 1

Enteroviruses



The third key contribution was the development of methods for production and application of the mass-produced cell culture[35] required by virologists for analysis of individual and herd response to enterovirus infection. Mass-produced cell cultures were something new. They were made possible through a) use of antibiotics for control of contamination, b) trypsin to disperse cells for preparation of single cell and monolayer cultures on glass, and c) cell-dispensing apparatus to provide replicate cultures. Human and animal cells in primary or continuous culture have largely replaced animals and embryonated eggs for isolation of viruses, assay of antibodies and many other purposes. The specific diagnosis of poliomyelitis and of many other previously unclassified infectious diseases is accomplished readily by cell-cultural methods. Today, commonly a virus is isolated and recognized by ability to affect and destroy susceptible cells in culture[66] while the capacity of homotypic antibody to neutralize cytopathogenic potential of the virus permits identification of isolates. This development stems from accomplishment by Enders, Weller and Robbins[17] of the cultivation of poliovirus in human cells of nonnervous origin, and their emphasis of earlier observations that cellular destruction and pH change could be employed as indicators of virus infection.[23, 24] Virologists were encouraged to examine the potentialities of the cultivated cell as a test host; the end result, as you know, has been the incorporation of cell-culture technic into virology as a primary and indispensable tool. It was the use of tissue cultures for isolation of polioviruses from fecal specimens that resulted inadvertently in the discovery of the enteric cytopathogenic human orphan (ECHO) family of viruses, and to most members in the tribe of enteroviruses.

The enteroviruses are host-specific pathogens found transiently in the indigenous orointestinal microbial population of men,[9] of monkeys,[22, 26, 27, 28] cattle,[44] swine,[61] and probably other animal species. These viruses are similar in size, resistance to ether, cytopathogenic effect, seasonal incidence, epidemiologic pattern and modal disease spectrum. Of the enteroviruses, the ECHO, Coxsackie and polioviruses are infectious for man. The ECHO family includes 24 antigenically distinct viruses, with several others under study. The properties shared by this family of viruses have been summarized by the Committee on the ECHO Viruses of the National Foundation[8] as follows:

> "(i) They are cytopathogenic for monkey and human cells in culture . . . (ii) They are not neutralized by pools of the three types of poliovirus antiserum. (iii) They are not neutralized by antiserums for Coxsackie viruses that are known to be cytopathogenic in tissue culture, and they fail to induce disease in infant mice . . . (iv) They are not related to other groups of viruses recoverable from the alimentary tract (throat or intestine) by inoculation of primate tissue culture, such as herpes simplex, influenza, mumps, measles, varicella, and the ARD (adenovirus) . . . or APC . . . group. (v) They are neutralized by human gamma globulin and by individual human serums; this indicates that they infect human beings."

ECHO 10, an extraordinary immunotype,[9] differs from other types in size, cytopathogenic effects, inclusion of certain mouse pathogenic strains within the type, and capacity to produce respiratory disease in chimpanzees and children.

PATHOGENESIS AND CLINICAL FEATURES

Cumulative evidence from studies of enterovirus infections indicates that a) the oropharynx is the portal of entry, b) the intestinal tract is the primary site of multiplication, and c) feces the vehicle of excretion and spread. The spectrum of host response to infection by any enterovirus strain ranges from a) inapparent infection; b) nonspecific febrile illness; c) mild respiratory

TABLE 2

Clinical Features of Enterovirus Infections

Common
inapparent infection
mild indeterminant or febrile disease with or without gastrointestinal disturbance, and/or less commonly, symptoms referable to the respiratory tract
Extraordinary
exanthema (ECHO-2, 4, 6, 9, 14, 16; Coxsackie A-2, 4, 9, 16 and B-4)
enanthema
enteritis
hematuria
pleurodynia
herpangina
aseptic meningitis (ECHO-2, 4, 6, 9, 14, 16; Coxsackie A-2, 4, 9 and B-4)
Serious
paralysis
encephalomyocarditis
carditis

illness, exceptionally; and d) gastrointestinal disturbance (Table 2). The latter symptom is associated commonly with anorexia and abdominal pain and less commonly with vomiting and/or diarrhea.[50, 54] This spectrum of host response can include extraordinary aspects: rash and transitory hematuria with ECHO virus infection; pleurodynia with Coxsackie B virus infection; herpangina and vesicular exanthema with Coxsackie A virus infection. Myocarditis, aseptic meningitis, and/or paralysis are seen as serious complications. The exceptional manifestations are attributed to hematogenous transfer of virus from primary sites of multiplication to secondary sites. While this wide range in host response may be observed in any group of patients infected with any one enterovirus, clinical response commonly reflects family and immunotype of the offending virus. Recognition of a systemic infection that may include in its manifestations paralysis, aseptic meningitis, carditis and/or maculopapular rash resembling that of rubella, usually is beyond a clinician's diagnostic acumen (Table 3). Laboratory aid is essential to a specific diagnosis. For example, clinical paralytic poliomyelitis results commonly from infection by a poliovirus, but clinical and pathological acute anterior poliomyelitis may result exceptionally from infection by other enteroviruses.[62]

Paralysis although acceptably pathognomonic for poliovirus infection, has been shown as already mentioned to result exceptionally from infection by other enteroviruses. Viruses reported to cause paralysis are Coxsackie B3 and B4;[21] B5;[21] Coxsackie A7,[51] and A9;[21] ECHO virus types 2,[63] 4,[21] 6[25] and 16.[21] Occurrence of less than 15 reported cases in a decade of prevalent disease suggests paralysis may continue to be regarded as singular to poliovirus infection.

Acute myocarditis, pleurodynia, epidemic myalgia, aseptic meningitis, and/or minor febrile illness result from infection by viruses of the Coxsackie B group and occur concurrently in epidemic infection. The syndrome of epidemic pleurodynia, or Bornholm disease[10] was familiar to physicians long before discovery of the causative virus. The syndrome is characterized by an incubation period of from 2 to 12 days, abrupt onset of symptoms with excruciating chest

TABLE 3

Association of Enteroviruses with Human Disease

Enteroviruses	Associated Disease
Polioviruses, Types 1–3	Summer febrile illness Aseptic meningitis Paralysis (flaccid to muscle weakness)
Coxsackie Viruses, Group A, Types 1–19	Summer febrile illness Aseptic meningitis (types A-2, 4, 9) Paralysis (?) (A-7, 9) Herpangina
Coxsackie Viruses, Group B, Types 1–5	Summer febrile illness Aseptic meningitis Paralysis (?) (types B-3, 4, 5) Pleurodynia Myocarditis or encephalomyocarditis in neonatal period and early childhood (types B-2, 3, 4)
ECHO Viruses, Types 1–24	Summer febrile illness Aseptic meningitis (types 2-6, 9, 14, 16) Paralysis (?) (types 2, 4, 6, 16) Summer rash (types 2, 4, 9, 16) Summer diarrhea of infants and children (type 18 and others)

pain substernally or unilaterally, and/or less frequently abdominal pain simulating an acute abdomen, and a febrile illness lasting from 7 to 14 days. The symptoms are attributed to focal and diffuse myositis. When pleurodynia occurs in a group or community other manifestations are found. Like newborn mice, infants may suffer focal and diffuse myocarditis from B-virus infection, focal fat necrosis,[3] necrosis of the adrenal cortex, liver necrosis and encephalomyelitis. Myocarditis with high mortality rate has occurred sporadically or epidemically in nurseries. The source of infection has been ascribed to minor illness of either mother or baby's attendant. Two epidemics involving 13 infants and seven deaths have been reported from Southern Rhodesia and the Transvaal;[15, 29, 36, 45, 60, 64] four newborn infants succumbed to myocarditis in Holland;[71, 72] a single case was reported from Hawaii[14] and, finally, two of these instances of myocarditis in infants from Boston were ascribed to intrauterine infection.[3, 34] Several of these infants suffered meningoencephalitis concurrently. Coxsackie B viruses immunotypes 2, 3 and 4, were the causative agents.

Aseptic meningitis, the manifestation most commonly observed, is characterized by acute onset with meningeal involvement, pleocytosis, absence of bacteria, short and benign course, and absence of pyogenic parameningeal infection. This syndrome has long been associated with a variety of viral infections, and with tuberculosis and leptospirosis as well. Aseptic meningitis occurs commonly as a complication of systemic enterovirus infection (Sabin, to be published) whether sporadic, endemic or epidemic in extent. It is doubtful whether a single type of enterovirus has escaped indictment as a causative agent of aseptic meningitis. With aseptic meningitis in epidemic form, evidence of causation is direct and unequivocal. This category of aseptic meningitis has been recognized since 1950, through use of cell-culture technics for etiologic distinction of human illnesses previously grouped by clinical diagnosis or epidemiologic evidence.

Aseptic meningitis in epidemic form not uncommonly is accompanied by an exanthem. The rash occurs commonly on face and upper trunk as blotchy, maculopapular, rubelliform or occasionally petechial lesions. At first sight, petechial rash and meningitis can misleadingly suggest meningococcemia.[15, 29] The enteroviruses which have been reported commonly as causative of febrile illness, aseptic meningitis and/or rash are ECHO types 2, 4, 6, 9, 14 and 16; Coxsackie A-2, 4 and 9 and Coxsackie B-4. Exanthem has been observed in patients ill from infection with viruses of ECHO types 2, 4, 6, 14 and 16 and Coxsackie types A-2, 4, 9 and B-4. Of these viruses, in recent years ECHO 9 has proved the principal offender.

Widespread outbreaks of ECHO 9 virus infection characterized by aseptic meningitis, rash, or both are known to have occurred in Britain in 1954 and 1955,[4, 43, 70] in Italy in 1955,[1] in many parts of Western Europe and Canada in 1956 and 1957,[3, 19, 21, 39, 48, 56, 59, 65, 74] and in Canada, Wisconsin,[57] Minnesota[26, 49] and other North Central and Middle West States in 1957. Forty thousand cases are estimated to have occurred in the Milwaukee area[57] and more than 220,000 out of a population of 1,240,000 in the Minneapolis-St. Paul area.[57] ECHO type 9 exanthem was observed easily on the face and upper trunk as a blotchy, maculopapular rubelliform or occasionally petechial rash. The rash led to a clinical diagnosis for some patients of rubella and for others of meningococcemia. The incubation period ranged from 3 to 6 days, but it was occasionally longer. The onset was abrupt or insidious, resembling at first a minor illness. Fever, headache, rash, nausea, vomiting, stiff neck or back, sore throat and myalgia were accompanied by a biphasic fever of from 2 to 15 days with average duration of 4 to 9 days. The peripheral white count was within normal limits, but exceptionally elevated to 15,000. The spinal fluid count was usually below 1,000 with neutrophils at first and replacement by mononuclear cells. Pleocytosis sometimes persisted for several weeks. The rash in contradistinction to that in roseola or Boston exanthem (ECHO 16) disease persisted during the course of the fever. A group A Coxsackie virus antigenically related to A-16 was held responsible[52] for an epidemic near Toronto which was characterized by a clinical syndrome of fever, ulcerative pharyngeal lesions and vesicular exanthem. The Toronto illness was mild, of short duration and not accompanied by meningitis or other complication.

ECHO 4 and ECHO 6 virus diseases have been reported predominantly as epidemic aseptic meningitis. ECHO 4 meningitis was observed without rash in Iowa[5, 42] and with rash in some patients in Sweden.[21] ECHO 6 has been proved many times a causative agent of aseptic men-

ingitis[13, 20, 35, 40, 73] with evidence for a widespread epidemic in New York State.[22, 75]

ECHO 16 virus infection was described from Boston and Pittsburgh as Boston exanthem disease.[46, 47] The rash did not appear until several hours to 2 days after the temperature returned to normal, resembling in that way roseola infantum.

ECHO 18 virus disease has been reported as an outbreak of summer epidemic diarrhea of newborn infants.[16]

Aseptic meningitis from Coxsackie infection simulates ECHO virus meningitis. Coxsackie B-1, 2, 3, 4 and A-2, 4 and 9 viruses have been associated with sporadic cases of aseptic meningitis; Coxsackie B5 and a group A Coxsackie virus antigenically related to A-16 have resulted in infections in epidemic form. Epidemic Coxsackie B5 infection occurred in the summer of 1956 in the states of Minnesota[67] and Iowa[4] and has recurred in successive summers. This history is remarkable principally because the causative virus had not been encountered in human illness since its discovery in 1952 by Steigman.[61] Coxsackie A-16, or closely related virus, was held responsible for an outbreak of febrile illness with pharyngeal lesions and exanthem but, remarkably, none of the patients had aseptic meningitis.[52]

DIAGNOSTIC AND EPIDEMIOLOGIC STUDIES

The evolutionary history of the enteroviruses is evidence that the rapidly expanding field of cellular biology has applied as well as pure scientific value. Practical applications of cell culture are helpful to the physician and to the epidemiologist (and indirectly to the average citizen) for isolation of viruses and accurate assessment of individual or group immunity. By use of cell cultures, a single laboratory may amass enough data to contribute materially to knowledge of epidemic human disease. Each year since 1946 we have performed laboratory studies, as have others, of human illnesses categorized by clinical diagnosis as poliomyelitis and/or aseptic meningitis. These studies[49, 67, 68, 69] over a 10-year period resulted in identification of more than 1,000 wild strains of poliovirus from as many patients. These 1,000 strains were recovered from only about 70% of all patients hospitalized with a clinical diagnosis of poliomyelitis. We failed each year to recover a poliovirus from 20 to 40% of the group. Many of these patients yielded cytopathogenic agents other than poliovirus. For example, the data for 1955 (Table 4) show that 64% of the paralytic group yielded virus in contrast to the surprisingly low percental number of 12 from the nonparalytic category; the total percental recovery was 33%. These data become more understandable when the results of all virus isolations for the period of 1955 through October 1958 are reviewed (Table 5). In 1956 the same program for identification of wild cytopathogenic viruses associated with syndromes diagnosed clinically as poliomyelitis or aseptic meningitis resulted[67] in etiologic characterization of an

TABLE 4

Poliomyelitis Virus Isolations from Stools of Cases Occurring Between April 1, 1955 and Dec. 31, 1955 and Distributed According to Clinical Type of Disease—Minnesota 1955

Clinical type	Number of patients	Poliovirus type*			Positive	
		1	2	3	total	%
Spinal paralytic	126	58	8	13	79	62.7
Bulbar	19	9	3	4	16	84.2
Bulbospinal	33	13	2	4	19	57.6
Total paralytic	178	80	13	21	114	64.0
Non-paralytic	263	17	8	6	31	11.8
Total	441	97	21	27	145	32.9
(% by type)		66.9	14.5	18.6	100.0	

Prepared by L. M. Schuman, M.D.
* Unduplicated

TABLE 5

*Virus Isolations in Minnesota 1955–1958**

	1955	1956	1957	1958
Poliovirus (all types)	175	47	9	12
Coxsackie A9	0	2	4	2
B2	0	0	2	0
3	0	6	1	2
4	0	0	1	2
5	0	60	18	109
ECHO 1		0	6	0
6		0	2	1
9		0	149	5

* Virus identification made in laboratories of the Dept. of Bact., Univ. of Minn. and/or Minnesota Dept. of Health.

TABLE 6

Viral Diseases of the Central Nervous System, Minnesota

Diagnosis	1955	1956	1957	1958*
Paralytic poliomyelitis	218	67	31	20
Nonparalytic poliomyelitis	292	82	33	2
Coxsackie B5 aseptic meningitis		75	23	109
ECHO 9 aseptic meningitis			228	5
Western equine encephalitis		2		6
St. Louis encephalitis		3		
Lymphocytic choriomeningitis	10	11	3	2
Mumps meningoencephalitis	52	25	17	52
Aseptic meningitis, etiology undetermined	47	78	401	387

* Data current as of Oct. 20, 1958. Data provided by courtesy of Dr. Herman Kleinman, Chief, Section of Chronic Diseases, Div. of Disease Prevention and Control, Minnesota Dept. of Health.

epidemic of aseptic meningitis as representing infection by immunotype B5 Coxsackie virus. Of 115 patients studied, 60 yielded Coxsackie B5 virus. In 1957 our study of epidemic aseptic meningitis demonstrated ECHO virus type 9 as the causative agent and in 1958, the current year, the predominant virus again has been Coxsackie B5. For the period 1955 to 1958, a summary of virus isolations in relation to diagnoses (Table 6) indicates that in Minnesota poliovirus is being replaced as the agent responsible for central-nervous-system disease of viral origin. Aseptic meningitis in Minnesota predominantly results from infection by viruses of Coxsackie B5, ECHO 9 and mumps in its meningoencephalitic manifestation. The viruses of lymphocytic choriomeningitis, Western and St. Louis encephalitis, and the enteroviruses Coxsackie B2, 3 and 4 and ECHO 1 and ECHO 6 were responsible for a few cases. Thus, without facilities for adequate testing made available by cell-culture diagnostic methods, the identity of each of a variety of causative agents responsible for aseptic meningitis would have been submerged in the amorphous category, "nonparalytic poliomyelitis" and recurrent opportunities to examine a series of human disease entities in relatively pure form would have been lost.

SUMMARY

We now know that human enteroviruses are a) *cosmopolitan in distribution*, being found in all parts of the world; b) *seasonal and temperate*, with occurrence limited principally to the summer months, and to tropical and subtropical areas and health resorts; c) *democratic in practice*, being causative agents of inescapable afflictions of man; d) *commonplace* in their selection of habitat, persisting under natural conditions as transient occupants of the orointestinal tract; e) *hardy*, making easy the problem of transfer to a new host by simple human association; f) *productive only of misery and ill health*, as evidenced each year by summer grippe and diarrhea, pleurodynia, paralysis, aseptic meningitis and myocarditis; and, finally, g) *eagerly ambitious* as shown by progressive displacement of the polioviruses as causative agents of viral disease of the central nervous system.

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A STUDY OF PIGMENTATION AND OTHER CHANGES IN THE LIVER IN MALARIA

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Hepatic involvement is an important aspect of malaria and presents several interesting problems. In 1924 Sinton and Hughes made the first reported study on liver function in malaria, using the levulose tolerance test. They found that abnormal tolerances occurred in almost all cases of hepatocellular jaundice caused by malarial parasites. Since that time numerous studies on liver function in therapeutic malaria have been carried out (Kopp and Solomon, 1943; Fredrick and Hoffbauer, 1945). These have shown that some degree of hepatic dysfunction often occurs during an acute attack of malaria. In 1946, Carter and McLagan used the serum colloidal-gold test and the thymol turbidity test to study hepatic dysfunction in 35 cases of *Plasmodium vivax* malaria. In the first test 92 per cent, and in the second test 80 per cent, of the specimens showed positive reactions. While the complete pathogenesis of malaria remains to be elucidated, it is generally accepted that the anemia which occurs during the course of the disease is one of the most important factors (Meagraith, 1948; Horner-Andrews, 1948; Boyd, 1949).

In addition to research on hepatic dysfunction, studies of the nature, toxic effect and production of malarial pigment have been made (Anderson and Morrison, 1942; Rigdon, 1945). Taliaferro and Mulligan (1937) found that malarial pigment can be seen only in Kupffer cells and never in parenchymal cells. During acute attacks this pigment appears as fine granules in Kupffer cells throughout the liver, lining sinusoid and sinuses in all parts of the lobules. Meagraith (1948) found that the amount, shape and colour of the pigment granules in the liver varied with clinical history, species of parasites and other factors.

EXPERIMENTAL

The aims of the present study were:

1. To investigate hepatic involvement in malaria by: (a) determination of hepatic dysfunction by means of the thymol flocculation test and the iodine test; (b) study of the relation of hepatic dysfunction, pathology of the liver and degree of parasitemia;

2. By studying malarial pigment in the liver to: (a) ascertain the percentage of malarial cases showing malarial pigment in the liver; (b) discover the earliest appearance of pigment and how long it remains in the Kupffer cells.

1. Study of Hepatic Involvement In Malaria

MATERIALS

Twenty-five cases of malaria were admitted to the department of medicine, Bhumibol Adulyadej Hospital, Bangkok, Thailand, during 1956 and 1957. Diagnosis of these cases was based on clinical picture and demonstration of parasites in peripheral blood. All were males, of whom 18 were soldiers between 17 and 28 years of age. The remaining seven, civilians, were from 24 to 52 years of age. All had uncomplicated malaria.

Residence of patients. All the service men lived at Donmaung, near Bangkok Airport which is comparatively free from malaria, but four of them had been in areas where malaria commonly occurs. Five of the seven civilian cases lived in Bangkok and the others were employed in infective areas. All patients remained in the hospital during the study of their cases.

METHODS

Clinical observations of cases. Physical examination and examination of blood for malarial parasites were made in all cases on day of admission. Estimations were made daily in cases with enlarged spleen and liver. Parasites per 1,000 rbc were counted every 4 hours after beginning treatment until they ceased to appear in peripheral blood. The day of the disease when parasite count was recorded in each case was the day on which treatment was begun (Table 1).

Treatment. All patients were treated on the first day of clinical diagnosis. The period of clinical symptoms varied from 3 days to 1

TABLE 1

Results of study of 16 cases of Plasmodium falciparum *infection and 9 cases of* Plasmodium vivax *infection*

No	Age (yrs)	Liver Function tests*			Malarial pigment†		Number of parasites/ 1,000 rbc	Day of R and parasite counts recorded	Enlargement of	
		Thymol flocculation test	Iodine test	Day test performed	Result	Day biopsy performed			liver	spleen
P. falciparum										
1§	32	2+	neg	5	4+	8	100	5	neg	neg
2§	24	2+	neg	30	2+	30	100	30	2 f.b.‖, tender	neg
3§	23	—	—	—	4+	—	80–100	—	—	—
4§	28	2+	neg	5	neg	6	80–100	5	2 f.b.	1 f.b.
5§	20	1+	neg	7	2+	7	80–100	7	neg	2 f.b.
6§	23	4+	2+	7	2+	12	50–60	7	neg	just palpable
7	22	3+	neg	8	1+	22	150	8	neg	neg
					trace	4 mo.				
					scanty	6 mo.				
8	25	2+	1+	5	1+	12	5	5	neg	neg
					scanty	3 mo.				
9‡	32	1+	4+	5	trace	10	5	5	neg	neg
10‡	24	3+	2+	8	neg	24	750	8	3 f.b., tender	neg
		3+	neg	24						
11	30	neg	3+	5	1+	7	40–50	5	neg	neg
12	26	3+	neg	9	trace	14	100–150	9	neg	neg
13	26	3+	neg	6	trace	12	40	6	neg	neg
14	28	3+	1+	3	1+	6	100	3	just palpable	2 f.b.
15	26	2+	1+	3	neg	10	100–150	3	3 f.b., tender	2 f.b.
		2+	neg	9						
		neg	neg	52						
16	22	3+	neg	—	biopsy not done		80–100	—	—	—
P. vivax										
17§	24	2+	neg	6	neg	6	60–80	6	neg	3 f.b.
18§	21	1+	neg	—	1+	6	20	—	neg	just palpable
					very few	6 mo.				
19§	52	2+	2+	14	3+	18	20–40	14	neg	2 f.b.
20	28	3+	neg	5	trace	12	10	5	neg	neg
21	17	2+	neg	7	trace	14	40–50	7	neg	neg
22	17	2+	1+	5	2+	7	15	5	neg	neg
23	22	2+	1+	6	1+	10	80–100	6	neg	2 f.b.
					trace	6 mo.				
24	22	3+	1+	6	1+	6	150–200	6	2 f.b., tender	3 f.b.
		2+	neg	20						
25	32	neg	neg	9	biopsy not done		10–20	9	neg	neg

* Plus signs (1+, 2+, etc.) indicate intensity of reaction.

† 1+ = pigment appeared as fine granules in some sinusoids.
2+ = pigment is more than 1+ and granules are rather thick.
3+ = pigment appeared in almost every sinusoid.
4+ = pigment appeared in every sinusoid, with thick granules.

‡ Jaundice occured in these two cases.

§ Cases of recurrent or long standing malaria.

‖ f.b. = finger breadths.

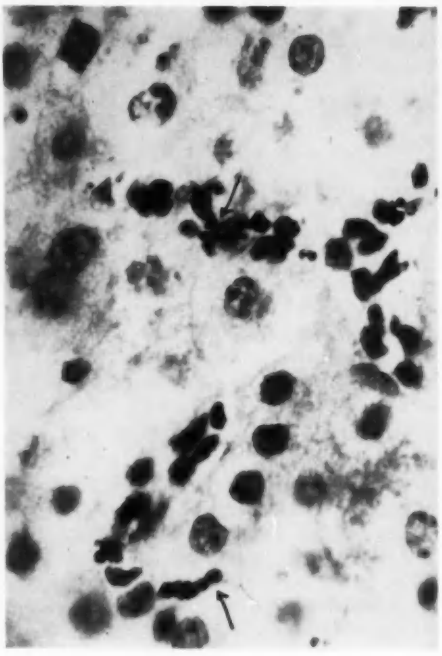

Figure 1. Case 1, Table 1. Malarial pigment in liver (note the great amount and thick granules). Liver biopsy was done on the 8th day after 1st clinical attack.

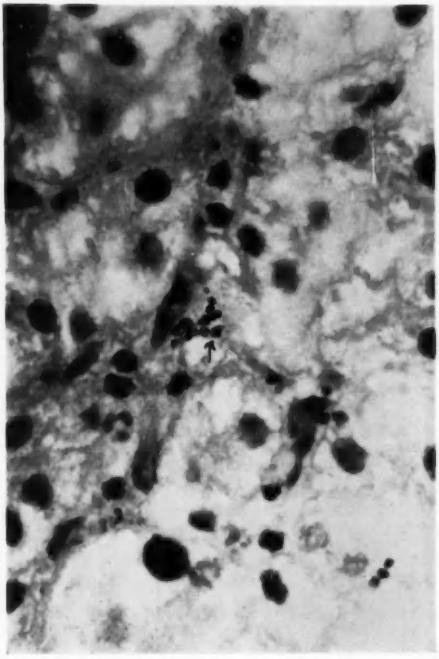

Figure 2. Case 23, Table 1. Malarial pigment in liver. Biopsy was done on the 12th day after the first clinical attack.

month before treatment. The drugs used were: (1) pyrimethamine given in 50-mg single doses in moderately severe cases (i.e., in cases of *P. vivax* infection and pamaquine maphthoape M.S. 8, given for the prevention of relapse, especially in cases showing gametocytes in the peripheral blood. (2) Chloroquine phosphate U.S.P., was given in 250-mg tablets; ten tablets in severe cases that did not show a satisfactory result from treatment with pyrimethamine. (3) Quinine hydrochloride U.S.P. and quinacrine hydrochloride U.S.P. (parenterally) were given in only one case of cerebral malaria.

Test used for Study of Hepatic Dysfunction

(*1*) *Thymol flocculation test.* The solution was prepared according to the McLagan method. By using 6 ml of solution plus 0.1 ml of serum, the result could be read within 48 to 72 hours. Kunkel and Hoagland (1947) found that the precipitation which occurred in this test resulted from a reaction between disintegrated lipids in serum and beta and gamma globulin; the last being increased in most cases.

(*2*) *Iodine test.* The solution was prepared according to the method of Mallen, *et al.*, (1950). By mixing one drop of solution and one drop of serum on the slide, the result could be read immediately. Shaper (1953) and Veranuvatti, *et al.*, (1954) studied this test and explained that, besides an abnormal ratio of albumin and globulin in serum, which is important in producing the precipitation (as described by Mallen), an increase of gamma globulin, which was found in most cases, may also be an important factor in this positive reaction.

RESULTS

Thymol flocculation test. Of 24 cases, 22 (92%) showed a positive reaction to this test, as follows: +, three cases (14%); ++, ten cases (45%); +++, seven cases (32%); ++++, two cases (9%).

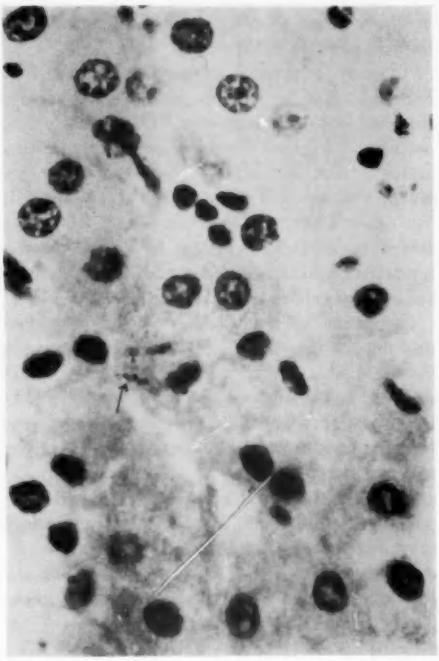

Figure 3. Case 23, Table 1. Malarial pigment in liver 6 months later.

Positive reactions occurred in 14 (93%) of 15 cases of *Plasmodium falciparum* infection and in eight (89%) of nine cases of *P. vivax* infection. Repeated examinations were made in three acute cases; they resulted in our finding that the change was temporary and lasted only 7 weeks after the first positive reaction.

Iodine test. Of 24 cases, 11 (46%) showed positive reactions as follows: +, six cases (55%); ++, three cases (27%); +++, one case (9%); ++++, one case (9%).

Of 15 cases of *P. falciparum* infection seven (47%), and of nine cases of *P. vivax* infection four (44%), showed positive reactions. Using three acute cases as in the thymol flocculation test to make repeated examinations it was found that the change was temporary and lasted only from 1 to 2 weeks during an acute attack of infection.

Clinical evidences of hepatic involvement. In our series, five (33%) of 15 cases[1] of *P. falciparum* malaria showed acute hepatic enlargement with tenderness during infection. This symptom subsided when antimalarial drug was given. Two cases with jaundice were noted in this group (cases 9 and 10, Table 1). Of nine cases of *P. vivax* malaria only one showed clinical hepatitis. In all these instances the parasite counts were over 100/1,000 rbc.

In the above 24 cases clinical hepatitis showed in only six. All of these six cases and 16 (89%) of 18 without hepatitis, reacted positively to the thymol flocculation test. When the iodine test was used, four of the six cases with evident hepatitis and seven of the 18 cases without evident hepatitis reacted positively (67% and 39%, respectively). Only one case (case 25) reacted negatively to both tests.

Degree of parasitemia and hepatic dysfunction. As shown in Table 1 (cases 5,6,8,13,16,19,20) degree of hepatic dysfunction did not necessarily depend upon numbers of parasites in the peripheral blood. This shows that there was no relation whatever between degree of parasitemia and hepatic dysfunction.

COMMENT

The thymol flocculation and iodine tests were used because the positive reaction of these tests indicates some change in serum protein. Since the liver influences protein metabolism, in cases of hepatic impairment some change in serum protein usually occurs (Veranuvatti, *et al.*, (1956). According to the above results, by using the thymol flocculation test, all cases with clinically evident hepatitis and 89 per cent of cases without clinically evident hepatitis showed a positive reaction. The results of this test therefore, showed little difference whether there was clinically evident hepatitis or not. With the iodine test, 67 per cent reacted positively in cases with clinically evident hepatitis; whereas only 39 per cent reacted positively in cases without clinically evident hepatitis. This indicates that patients having clinically evident hepatitis are more likely to react to the iodine test than those without such hepatitis while the thymol flocculation test produces positive reactions in both groups. It should be noted that the number of our patients who reacted positively to the thymol flocculation test (92%) is rather high when compared to the results of Carter and McLagan (1946), who found that 80 per cent of 35 cases infected with *E. vivax*, showed a positive reaction.

[1] Total cases were 16; one case (case 3) was deleted at this point in the study because of incomplete data.

While 28 per cent of the series had clinical symptoms of hepatitis, 92 per cent and 46 per cent of the series showed positive reactions to the thymol flocculation test and iodine test, respectively. This indicates that hepatic dysfunction commonly occurs in malaria though it may not be clinically evident. Our work also showed that although the species of parasites and degree of parasitemia may play important roles in causing hepatitis, the degree of hepatic dysfunction observed was not related to the above two factors. This result was the same as that obtained by Kopp and Solomon (1943) in nine cases of *P. vivax* infection in which no relationship was observed between degree of parasitemia and hepatic dysfunction.

Comparison of sensitivity of these tests. The thymol flocculation test more commonly showed a positive reaction in malaria than the iodine test. However, we could not find any relation between the degrees of positivity to these two tests. Our results agree with those of Veranuvatti, *et al.*, (1954) who observed these tests in 96 cases and could find no relation between them.

2. Study on Malarial Pigment in the Liver

MATERIALS

These were the same as in Part 1.

METHODS

Liver biopsies were done by transthoracic approach in 23 cases of malaria by using a Vim Silverman needle. The liver tissues were fixed in 10 per cent unbuffered formalin and stained by the hematoxylin-eosin method.

Liver biopsies were also done in five control cases that had no malaria at the time of biopsy and had never been attacked by malaria. The liver tissues were fixed in 10 per cent unbuffered formalin and stained by the hematoxylin-eosin method.

Intervals between first clinical attack and time of liver biopsy varied from 6 days to 1 month.

RESULTS

In the five control cases neither malarial pigment nor any structure that resembled malarial pigment was found in the liver.

In cases studied, malarial pigment was found in Kupffer cells in 19 (83%) of 23 cases. Eleven of these cases (57.9%) were *P. falciparum*; 8 were *P. vivax* (42.1%).

Time of occurrence of pigment in the liver. Pigment could be seen as early as 6 days after the first clinical symptom (liver biopsy was performed on that day); whereas the shortest period of clinical attack, before treatment was given, was about 3 days (case 14, Table 1). In the other cases the shortest period of clinical attack varied from five days to 1 month before treatment; and liver biopsy was done in each of these cases a short time after treatment. (Fig. 1.)

The period of time during which pigment was still found after the first deposition. In three of four cases studied (cases 7,8,18,24), which showed a moderate amount of pigment (1^+), the pigment was still be seen 6 months later but the amount was very small when compared to what was observed at the first biopsy. In one case that showed a smaller amount of pigment than the above 3 cases, pigment had almost disappeared 3 months later. (Figs. 2, 3.)

Relation between amount of pigment and chronicity of the disease. A considerable amount of pigment was shown in seven of 23 cases; five infected by *P. falciparum* (cases 1,2,3,5,6) (34% of *P. falciparum* cases) and two infected by *P. vivax* (cases 19,22) (25% of *P. vivax* cases). Six of these cases (86%) were part of a group of nine cases of recurrent malaria (Table 2); one (case 2, Table 2) had a history of long-standing malarial infection and five (cases 1,3,5,6,19, Table 2) had been periodically attacked by malaria. Only one (14%) of these cases (case 22, Table 1) showed no sign of long infection. From the above results it may be concluded that the amount of pigment in the liver depended upon the chronicity of the disease.

Relation between amount of pigment and degree of parasitemia. There were seven cases which showed an inverse relationship between amount of pigment and degree of parasitemia (cases 10,12,13,15,16,19,22, Table 1). The duration of the attack, the time when liver biopsy was performed and species of parasites may be responsible for the different amounts of pigment in these cases. In order to eliminate, as far as possible, error due to these factors in determining the relation between amount of pigment and degree of parasitemia, those patients with almost the same duration of attack, interval between beginning of treatment and liver biopsy, and infected with the same species of

TABLE 2

Amount of pigment in nine chronic cases

Case	Parasite species*	Liver function tests			Pigment and Pathology		History
		Thymol flocculation test	Iodine test	Day tests performed, R given	Result	Day of biopsy	
1	f	2+	neg	5	4+ cloudy swelling	8	Patient was employed near a malaria focus, had frequent malaria.
2	f	2+	neg	30	2+	30	Same as 1, f; present illness was 1 month prior admission.
3	f	—	—	—	4+	—	Same as 1, f.
4	f	2+	neg	5	neg degeneration	6	One malarial attack 6 months ago.
5	f	1+	neg	7	2+	7	Repeatedly attacked by malaria; last attack about 1 month ago.
6	f	4+	2+	7	2+ cloudy swelling	12	Same as 5, f.
17	v	2+	neg	6	neg degeneration	6	Same as 4, f.
18	v	1+	neg	—	1+	6	Same as 5, f.
19	v	2+	2+	14	3+	18	Patient lived in an infective area and had frequent malaria.

* f = *Plasmodium falciparum*; v = *Plasmodium vivax*.

parasites, were grouped together for comparison. For example, in cases 7 and 10 the duration of attack was 8 days before beginning of treatment, and biopsies were performed on the 14th day and 16th day of disease, respectively. In case 10, malarial pigment was absent, despite the large number of parasites that were found (as high as 750/1000 rbc); whereas in case 7, even though only 100 parasites were found in 1000 rbc, a moderate amount of pigment was present.

With respect to the long duration between beginning of treatment and liver biopsy, we might postulate that the pigment in case 10 was absent because it had been absorbed during this period of only 16 days. Even were this possible, some pigment should have been present, even though in very small amount, because follow-up study in four cases (cases 7,8,18,23) showed that some pigment can be seen as long as 3 to 6 months after the first deposition, even in cases where there was only moderate amount of pigment deposited.

Similarly, in cases 4 and 8 the treatment was begun on the same day (5th day) of disease. Malarial pigment was not seen in case four, despite the period of only 1 day between the beginning of treatment and liver biopsy; 100 parasites were found in 1000 rbc. In case 8, 1+ of pigment was present, even though the duration of treatment before biopsy was 7 days and very few (5/1,000 rbc) parasites were found in the peripheral blood. Similar results were seen in cases 17 and 22 and in cases 13 and 15. From these facts, we may conclude that there was no constant relationship between degree of parasitemia and amount of pigment in our study.

Relation between amount of pigment and degree of hepatic dysfunction. There was no constant relationship between amount of pigment and degree of hepatic dysfunction in cases 1,5,10,12, 13,21 (Table 1), as measured by the thymol flocculation test and the iodine test.

COMMENT

The above results show that 83 per cent of the cases studied had demonstrable malarial pigment in the liver. It is recognized that the use of unbuffered formalin in our studies might

introduce an error due to formalin pigment which is virtually indistinguishable in appearance from malarial pigment. We studied pigmentation in the liver in five control cases, using the same fixing solution, in order to find out how much error would be obtained by using unbuffered formalin. It resulted in our study that no visible formalin pigment was found in the liver of these five cases. From this result, we feel reasonably safe in saying that the use of unbuffered formalin solution in our laboratory for fixing the liver tissue, did not introduce significant error due to formalin pigment formation. However, considering that formalin pigment might have formed in minute amounts, we may point out that we did have five cases among 23 (21.7%) in our series which showed only traces of pigment. Admitting that these might represent error due to using unbuffered formalin the percentage error would not exceed 21.7 per cent, and was probably very much less. With regard to the species of parasite as a factor in producing varying amounts of pigment it was not possible to obtain definite results as the number of cases studied was small. However, in this study it was found that there was little difference between the amounts of pigmentation resulting from different species of infection (34%, 25%). It may be concluded, therefore, that the amount of pigment did not depend upon the species of parasites.

Our work agrees with that of Maegraith (1948) who found that the amount of pigment depended upon the frequency of attack, but our results differed from his regarding the relation between pigmentation and species of parasite.

Parasitemia and pigment. It can be seen in Table 1 that there was no relation between amount of pigment and degree of parasitemia. This result suggests that, although malarial pigment is a product derived from changes of hemoglobin in parasitized cells by the metabolism of the parasites, the amount of pigment does not depend absolutely upon the number of parasites in the peripheral blood or on the amount of hemoglobin in red cells attacked. There may be some factors which are more important, such as duration or chronicity of the disease. Our work also showed that, even in cases where there was a great number of parasites in the peripheral blood, pigment was not always found in Kupffer cells of that case. These findings are contrary to those of Gaskel and Millar, who found that there is a large quantity of pigment in cases of *P. falciparum* infection, especially in cases where there is a large number of parasites in the peripheral blood.

It was found that large amounts of pigment disappeared more slowly than small amounts. This indicated that the time required for pigment to disappear after beginning treatment depended upon the quantity of pigment in the liver at the time of beginning treatment.

Our failure in four of 23 cases to find malarial pigment in the liver might have been due to the fact that the amount of pigment was so small that it did not pass through the liver. In our study considerable amounts of pigment were found in cases of recurrent or long-standing malaria, and the period of time between beginning of treatment and biopsy in each case appeared to be responsible for the reductions in amount of pigment observed at the time of biopsy. We therefore conclude that the amount of pigment deposited in acute attacks varies with the duration of the attack but not at all with the degree of parasitemia.

The amount of pigment found at biopsy tends to vary with the amount of pigment deposited *before treatment was begun* and the *duration of treatment before biopsy was performed.* For example, treatment of cases 20 and 22 was begun on the fifth day; 2^+ of pigment was present when biopsy was performed on the seventh day (case 22) but only a trace of pigment was found at biopsy on the 12th day (case 20). Similarly, in cases 6 and 7 treatment was begun on the seventh and eighth days; 2^+ of pigment was found when biopsy was performed on the 12th day (case 6) and only 1^+ found at biopsy on the 22nd day (case 7). On the other hand, in case 19, treatment was not begun until the 14th day, permitting abundant pigment deposition, and these deposits were found in a biopsy performed only 4 days later. The above results indicate that the amount of pigment found in the liver depends on the duration or chronicity of the disease as well as the time when biopsy was performed after the beginning of treatment.

Our study showed that there is no relation between degree of hepatic dysfunction and amount of pigment in the liver. The results indicated that malarial pigment is not an important factor in causing hepatic dysfunction.

It confirmed the opinion of many workers that in the reticuloendothelial system this pigment is harmless, whereas in the circulation its toxicity is remarkable (Anderson and Morrison, 1942).

With regard to pathology, the liver of most of our patients showed some degree of cloudy swelling and hydropic degeneration. Three cases showed hepatic congestion. Central necrosis, which is a most significant hepatic change at autopsy in malaria, was not found in our patients. We also found that in some cases which showed only slight changes by the thymol flocculation test, pathological changes in the liver were more severe than in cases with high degrees of hepatic dysfunction. It should be noted that although the thymol flocculation test is useful to detect some degree of hepatic dysfunction, it can not show the degree of pathological change during the course of the disease.

Summary and Conclusions

The results of our studies of the liver in 25 cases of malaria were as follows:

1. The thymol flocculation test and the iodine test showed positive reactions in 92 per cent and 46 per cent of the series, respectively. The iodine test was more sensitive in cases of malaria with clinical hepatitis while the thymol flocculation test showed a positive reaction in almost every case of malaria (with or without clinically evident hepatitis). Correlation between the positive reactions of these two tests was not evident in our series.

2. In this series 28 per cent of cases showed clinical symptoms of hepatitis. The species of parasite and degree of parasitemia seem to be the important factors in causing the symptom. But hepatic dysfunction, which commonly occurred, did not depend upon either species of parasite or degree of parasitemia.

3. Of the series of cases 83 per cent showed malarial pigment in Kupffer cells. Of these infections, 63.2 per cent were caused by *Plasmodium falciparum* and 36.8 per cent by *Plasmodium vivax*.

4. We found that pigment could be demonstrated in the liver as early as 6 days after commencement of the clinical attack and as long as 6 months after beginning treatment. The time of disappearance of the pigment depended upon the amount of pigment deposited at the beginning of infection. The amount of pigment in the liver depended on the duration or chronicity of the disease as well as the time when biopsy was performed after the beginning of treatment.

5. A constant relation between hepatic dysfunction, amount of pigment, degree of parasitemia and pathological changes in the liver could not be demonstrated in our series of observations.

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THE COCKROACHES *BLABERUS CRANIIFER* AND *BLABERUS DISCOIDALIS* AS VECTORS OF *SALMONELLA TYPHOSA*

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The role of cockroaches as agents for the dissemination of enteric pathogens has been demonstrated by many investigators. So many of these organisms have been passed through the digestive tract of roaches with no apparent harm, that when an exception occurs it stands out. Macfie (1922), Macburney and Davis (1930), Wedberg, *et al.*, (1949), and Janssen and Wedberg (1952) have pointed out the failure to implant roaches with *Salmonella typhosa*. These reports suggested the presence in the gut of some lethal agent, or agents, specific for *S. typhosa* which does not appear to affect related *Salmonella* species.

Numerous references can be found in the literature relative to antibacterial agents in insects, and the present paper seeks to elucidate the nature of the lethal substance responsible for the reported findings with respect to destruction of the typhoid organisms in roaches.

MATERIALS AND METHODS

Adult females of the two species of tropical cockroaches, *Blaberus craniifer* Burmeister and *Blaberus discoidalis* Serville, were used indiscriminately. For controlled feeding of pathogens the roaches were mounted according to a method found useful (Fig. 1). The insects were fastened loosely to blocks of wood by means of aluminum wires. Feces were collected in sterile Petri dishes containing tetrathionate broth for the cultivation of *S. typhosa*, or the brilliant-green-sodium-taurocholate medium devised by Stokes and Osborne (1955) when growth of *S. enteritidis* was desired. The dishes were protected from contamination caused by splashing from the mouthparts and the thrashing legs of the roaches, with the aid of an aluminum "napkin" girdling the abdomen and attached to a block of wood with melted paraffin. The roaches were suspended from a Petri dish cover placed inside a large beaker, and the interior of the chamber was kept moist with about 100 ml of a 1:1,000 solution of mercuric chloride which also served as a disinfectant in the bottom of the beaker.

The mounted insects were fed a diet of skim milk, yeast extract (1.0%), and sucrose (0.5%). When the roaches were to be fed test organisms, the growth from the surface of 24-hour, standard, nutrient agar slants was washed off with the milk diet. Determination of the numbers of viable organisms per ml of the resulting suspension was accomplished by dilution plating in standard nutrient agar incubated at 37°C for 48 hours. Feeding of the roaches was carried out in the manner described by Wedberg and Clarke (1947). The *S. typhosa* culture employed in these studies was No. 6539 of the American Type Culture Collection, and the *S. enteritidis* strain was University of Connecticut No. 131 isolated in 1954 from a human stool.

To obtain a direct measurement of the pH of the digestive-tract contents of the roaches a micro pH meter was constructed according to an adaptation of the technic of Pierce and Montgomery (1935). The instrument was devised using a Beckman Model H2 pH meter with the glass electrode removed and replaced with a platinum wire. The wire was flamed, allowed to cool, and dipped into a slurry of quinhydrone crystals in acetone. A few crystals adhered to the wire. An acid-cleaned Pyrex capillary tube with a diameter of about 0.5 mm was dipped into mineral oil until the oil had risen several mm in the tube. The wall of the crop, midgut, or salivary sac was then punctured with the capillary tube, allowing the contents to rise into the tube. Parts of the alimentary canal were not removed from the roach during sampling.

Immediately after withdrawing the tube from the test area the end of the capillary was plunged into a block of KCl-saturated 3% nonnutrient agar which served to seal the sample from the air. The platinum electrode with adhering crystals was then inserted into the

Figure 1. Lateral view of *Blaberus craniifer* as mounted for controlled feeding

top of the capillary through the layer of oil into the sample, where the quinhydrone dissolved. The calomel electrode and the quinhydrone electrode were then connected by a saturated KCl bridge and the voltage was read on the galvanometer. This design is shown schematically in Fig. 2.

Aseptic precautions were employed in all experiments involved in isolating bacteria from the crop. Roaches were anesthetized in a stream of carbon dioxide, followed by dipping them into 1:1,000 tincture of Merthiolate for about 1 minute. Drying of the insects was accomplished in a sterile Petri dish, and all instruments employed were sterilized prior to use. The yeast extract medium employed consisted of yeast extract (0.3%), Tryptose (0.5%), Casitone (0.5%), sugars, if any, (0.5%), and distilled water.

To test the bactericidal action in vitro of roach-gut contents a layer of yeast extract was poured into a Petri dish and allowed to solidify. On top of this was added more agar seeded with *S. typhosa*. Before the top layer of medium solidified, sterile Penicylinders were inserted in such a manner that they rested on top of the hardened bottom layer and were embedded in the seeded top layer of agar. The gut was aseptically removed from the roach and divided into crop, midgut, and hindgut. Each segment was macerated and added to respective Penicylinders. Following incubation at room temperature for from 24 to 48 hours, zones of bacterial inhibition around the Penicylinders containing crop material were exhibited in positive findings.

Bacteriophage. In addition to the above experiments attempts were made to demonstrate bacteriophage activity that might account for the destruction of the typhoid organisms in roaches. These attempts were unsuccessful. This aspect of the problem, however, may warrant further attention.

RESULTS

S. enteritidis fed to roaches in the magnitude of three billion cells were recovered from the feces of some insects up to their death on the

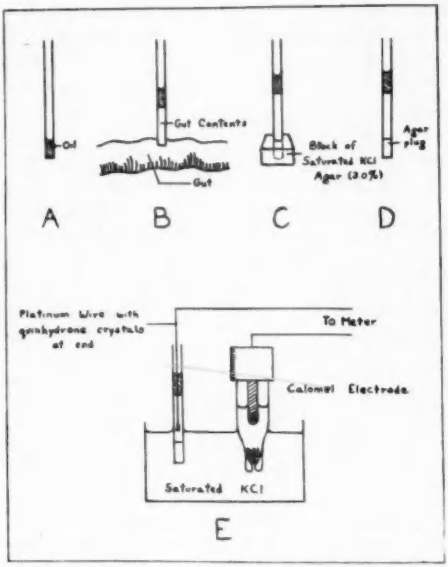

Figure 2. Method of making pH determinations with the microquinhydrone electrode: A, Pyrex capillary tube dipped into mineral oil; B, puncturing the gut with the tube, allowing rise of gut contents; C, plunging tube into KCL-agar; D, agar plug in bottom of tube; E, insertion of platinum wire with quinhydrone crystals on end into the test sample and connection to meter.

TABLE 1

Zones of inhibition with crop material from Blaberus *species against* Salmonella typhosa *and* Salmonella enteritidis

Roach	*S. typhosa* (Zone radius, mm†)	*S. enteritidis* (Zone radius, mm†)
1*	6	0
2	5	0
3	18	3

* The crops of the insects were divided into approximately equal parts and placed in Penicylinders on yeast-extract agar, without glucose (pH 5.4).

† Measured from edge of Penicylinder to periphery of zone of inhibition.

17th day following the infective feeding. On the other hand, *S. typhosa* fed in quantities approaching two billion cells were recovered but once from the excreta, and that was within the first 24 hours after feeding.

TABLE 2

Calculated pH values of various portions of the digestive tracts of roaches

Roach*	Saliva†	Crop	Midgut	Hindgut
1	6.6	3.9		
	6.5			
2	6.1	4.5		
	6.2			
3		4.5		
4		4.35		>7.7
5	6.3	4.4		6.9
6		4.3		
7	6.5	4.2		
8			6.4	7.7
9		4.6	6.3	
10		4.9		>7.7
11		4.6		

* Fed on Pablum Mixed Cereal diet.

† Indicates one salivary gland of the pair.

The size of the clear zone appeared to bear some relationship to the volume of gut contents placed in the cylinder. It was noted that such zones either did not appear or were very small when *S. enteritidis* was used (Table 1). Moreover, when agars of from pH 6.5 to 7.0 were used instead of pH 5.4, the zones were either absent or very small.

Determinations with the microquinhydrone electrode revealed low pH values in the crops of roaches, as distinguished from higher values for saliva, midgut, and hindgut contents (Table 2). The most common value was around pH 4.5.

Correlation between crop pH, ability of crop contents to produce zones of inhibition, and in vivo activity of the roaches against *S. typhosa* but not *S. enteritidis*, was indicated by studies on the survival of *S. typhosa* and *S. enteritidis* at various pH values (Table 3). These results indicate that *S. typhosa* could be destroyed or inhibited in the crop due to pH alone, but that *S. enteritidis* could not.

In an attempt to learn how long bacteria remain in the crop, roaches were fed milk containing sterile powdered carmine. At the end of 5 hours the carmine had progressed halfway down the midgut and by 12 hours the dye was detected at the beginning of the hindgut. The crops were still bright red at the conclusion of these time periods. Since the roaches had been fed a milk diet similar to that given with the

TABLE 3

Survival of Salmonella typhosa *and* Salmonella enteritidis *in yeast-extract broth at various pH values*

Organism	pH	Initial No.*	Interval (*hours*)			
			6	12	24	48
S. typhosa	5.30	176×10^3	275×10^4	255×10^5	410×10^5	400×10^5
	5.10	165×10^3	139×10^4	840×10^4	182×10^5	109×10^5
	4.79	143×10^3	620×10^2	570×10^2	360×10^2	650×10^1
	4.51	102×10^2	0	0	0	0
S. enteritidis	5.30	490×10^3	580×10^5	239×10^6	not done	not done
	5.10	560×10^3	280×10^5	144×10^5	not done	not done
	4.79	930×10^3	760×10^4	780×10^5	not done	not done
	4.51	340×10^3	260×10^3	127×10^5	218×10^5	227×10^5

* Viable cell numbers were determined by dilution plating in standard nutrient agar.

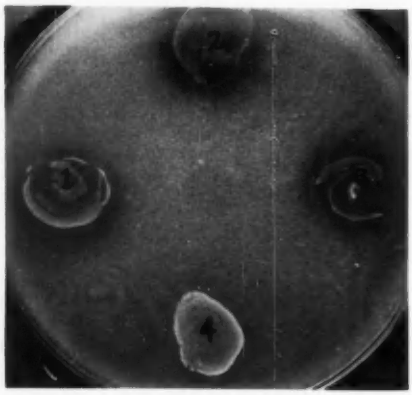

Figure 3. Inhibition of *Salmonella typhosa* by organisms resembling propionibacteria isolated from the crop. The medium is yeast-extract glucose (0.5%) agar.

Salmonella suspensions, it would appear that this prolonged exposure in the crop could result in the destruction of many of the test organisms fed with the milk diet.

Crop contents of roaches were smeared on slides and stained with polychrome methylene blue. Microscopic observation revealed occasional food particles and a fair number of yeasts, but the predominating organisms were bacteria with varying morphology. Dilutions of crop contents in tomato agar yielded standard plate counts of about 1×10^9 colonies per ml in roaches fed Pablum Mixed Cereal, while insects on Purina Dog Chow Checkers gave colony counts in the range of 77×10^6 per ml.

In further experiments, from 24 to 50 per cent of the crop bacteria were found to produce zones of inhibition against *S. typhosa* when pure cultures of crop isolates were smeared in small areas on seeded plates. When yeast-extract agar was used, zones were small or absent. When glucose was added to the medium, the zones were abundant. Using glucose agar, a pH of 5.3 exhibited more and larger zones than agars of pH 6.0 or 6.9. All of these results indicated that acid production by these crop isolates was responsible for the inhibitory activity against *S. typhosa* in the body of the cockroach.

The great majority of the zone-producing isolates appeared to be propionibacteria, similar to, but not identical with, *Propionibacterium freudenreichii*. Figure 3 shows some of the inhibitory zones produced by these organisms against *S. typhosa*.

DISCUSSION

The destruction of *S. typhosa* in the crop of roaches would depend not only upon the pH but also on the length of time the bacteria were exposed to the acid. At pH 4.9 in vitro there was only a slight decrease in bacterial population at the end of 6 hours, but at a pH of 4.51 there was total destruction of these organisms at the end of 6 hours. A pH of 4.5 or below is a common finding in the crop of roaches.

Carbohydrate-rich diets favor acid production. The saliva of cockroaches contains amylase which could produce maltose from ingested starches, and maltose is fermented in the crop by the organisms resembling propionibacteria.

The ceca and the midgut secrete invertase and lactase which could attack sucrose and the lactose in the milk diet with the formation of glucose, a sugar available to the zone-producing bacteria for acid production. Some of these bacteria are weak in their acid production when tested in pH 7.0 sugar media. Other organisms such as yeasts could help to maintain a low pH, the zone-producers contributing the extra hydrogen ions necessary to attain a pH of 4.5. This interpretation was indicated when larger zones of inhibition were produced on experimental plates with an initial low pH, than on plates at pH 7.0.

SUMMARY

A useful method of mounting *Blaberus* species is described which allows controlled feeding of test organisms. *Salmonella enteritidis* was excreted up to 17 days after being fed, while *S. typhosa* was recovered in only one instance, and that was on the first day. An in vitro test showed an antibacterial agent to be present in the crops of the roaches tested. Measurements with a microquinhydrone electrode revealed crop pH values ranging from 3.9 to 4.9 with the majority of the readings around 4.5. It was shown that typhoid cells are destroyed in vitro at pH values below 4.7, whereas *S. enteritidis* is more resistant. Organisms resembling *Propionibacterium freudenreichii* were isolated from crops and were shown to exhibit zones of inhibition on agar plates when tested against *S. typhosa*. The effect appeared to be due to acid production by the isolates. The in vivo antibacterial effect is probably due to the acid produced in the crop of the roaches through the action of microorganisms.

Attempts to demonstrate bacteriophage activity that might account for the destruction of typhoid organisms in roaches were unsuccessful.

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TRIMERESURUS ELEGANS (GRAY, 1849), THE SAKISHIMA HABU, A VENOMOUS PIT VIPER FROM THE RYUKYU ISLANDS

HUGH L. KEEGAN* AND KOZEN YOSHINO†

This paper is a two-part summary of existing information on a venomous terrestrial snake, *Trimeresurus elegans* (Gray, 1849), the Sakishima habu. This poorly known species occurs only on islands of Yaeyama-retto, Sakishima-gunto, about 240 miles south of Okinawa. Information presented here is particularly timely because of the current policy of the United States Civil Administration of the Ryukyu Islands in encouraging emigration of settlers from Okinawa to the more sparsely settled Yaeyama islands. Part I of this paper contains brief, illustrated descriptions of the Sakishima habu, and of a rare color variant, which may prove to be a new subspecies of *T. elegans*. Information is also given concerning effectiveness of available antivenins in neutralizing venom of the Sakishima habu. Part II contains the first published accounts of symptoms in man following bites by the Sakishima habu. Observations concerning treatment of bites by this snake are presented, and environmental factors which have a bearing on incidence of snake bite are discussed.

PART I

A pit viper of genus *Trimeresurus* has many small scales covering the dorsal surface of the head. This characteristic is illustrated in Figure 1. This condition serves to distinguish snakes of genus *Trimeresurus* from those of related genus *Agkistrodon*. Species of the latter genus have relatively large, symmetrically arranged plates covering the dorsal surface of the head. This is shown in Figure 2. Species of *Trimeresurus* occur throughout eastern Asia, but are most numerous in warm regions. Five species of the genus are found in the various island groups from Kyushu to the southern end of the Ryukyus chain. Most important of these, from a medical viewpoint, is *Trimeresurus flavoviridis flavoviridis* (Hallowell, 1860), the habu. This active, sometimes aggressive snake is found on Okinawa, and on Amami Oshima, as well as on adjacent small islands. Most snake bites reported from the Ryukyus are caused by reptiles of this species. Two subspecies of *flavoviridis* have been described, but these are not well known, and nothing has been published regarding potency of their venoms. These are *T. f. tokarensis* (Nagai, 1928) known only from Tokara-shima, an island now included in Kagoshima prefecture of Kyushu, and *T. f. tinkhami* Gloyd, 1955,[1] a subspecies which has been taken only on Kume-shima, a small island about 60 miles southwest of Okinawa. The himehabu, *T. okinavensis* Boulenger, 1892, occurs on Okinawa, and also on Amami Oshima. Throughout its range it is not as abundant as the habu and, while its venom is presumed to be potent, it is rarely listed as a cause of snakebite. The remaining species from this area, *T. elegans* (Gray, 1849) is found only on the islands of Yaeyama-retto in the southern Ryukyus. Although Maki[2] gave detailed descriptions of specimens he had examined, nothing has previously been published concerning habits, venom potency, and symptoms produced by bites of snakes of this species. Relatively few specimens of *T. elegans* have been deposited in museums of the world and, as recently as 1956, the collections of the United States National Museum contained no specimens of this snake. In view of this, it is of interest to note that Gray, the describer of *T. elegans*, thought that the type specimens of this snake, which had been deposited in the British Museum, might have been collected on the west coast of North America. These snakes had been collected during the voyages of a British vessel, the *Samarang*, in Asian waters. Stejneger[3] after studying the accounts of these voyages, concluded that the only likely place where the specimens could have been obtained was Ishigaki-jima. The collecting party from the *Samarang* stayed for some time on Ishigaki and obtained a variety of specimens.

Information presented here is based largely upon a study of specimens collected by the writer and Mr. Seiichi Toshioka on Ishigaki and Iriomote islands during September and November,

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† Shika; Ishigaki-jima, Ryukyu Islands.

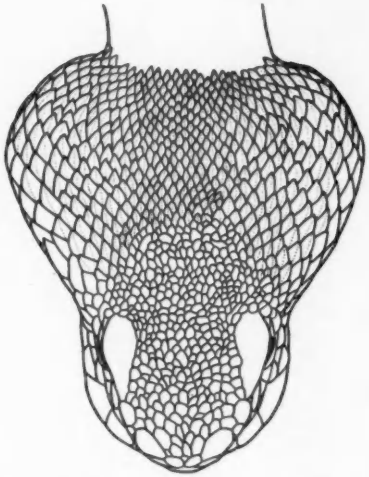

FIG. 1. Dorsal view of the head of a snake of genus *Trimeresurus*.

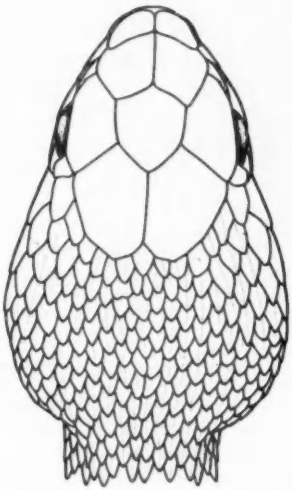

FIG. 2. Dorsal view of the head of a snake of genus *Agkistrodon*.

1955 and August and September, 1956. Most of these snakes were collected at night as they were crossing roads. The collector rode in a jeep, which was driven slowly along the road. When a snake was seen, the jeep was stopped and the collector ran ahead to catch the specimen while it was still in the lighted area of the road. The snakes were easy to see because their scales reflected light from a considerable distance. Most of these snakes did not coil to strike, but tried to escape. If touched they would often swing around to strike momentarily, but did not hold their ground in definite coils. Captured specimens were kept in cloth bags, and carried in well ventilated wooden boxes. They were given water at least every 48 hours by partially immersing the bags in buckets for 10 or 15 minutes. Some specimens were collected from daytime resting places in rocky areas by a local collector.

COLOR

The Sakishima habu is essentially gray above with a series of dark-brown, black-edged blotches along the dorsal mid-line. In some places these blotches are confluent, forming a zig-zag pattern. Smaller, lateral spots also occur along the entire length of the body. There are indistinct, dark markings on the top of the head, and there is a well-defined black band extending from the eye posteriorly through the angle of the jaw. Such a band is commonly possessed by members of genus *Trimeresurus* and is also exhibited by *Agkistrodon halys*, the mamushi. The undersurface is yellow with indistinct, dark markings. Many of the ventral plates are heavily suffused with gray, particularly at their lateral margins. Dorsal and lateral markings of very young specimens are more distinct than those of adults. These characteristics are illustrated in Figures 3 and 4.

SIZE

This is apparently a smaller snake than the Okinawan habu. The largest of eight specimens collected by one of the writers (Keegan) on Ishigaki measured 1,145 mm in length. In a series of 13 adult specimens whose measurements were given by Maki,[2] total length varied from 430 mm to 1,113 mm. Only two specimens in the series were over 1,000 mm in length. Islanders who were questioned stated that specimens as long as 6 feet or more were sometimes seen. If these do occur, they must certainly be rare. Exaggeration of size of specimens is not confined to fishermen.

SCUTELLATION

Within certain limits, numbers of scales, their arrangement, and their shapes and relative sizes are excellent taxonomic characteristics, and show

FIG. 3. Dorso-lateral view of a specimen of *Trimeresurus elegans*, the Sakishima habu

little variation among individuals of a species. Scutellation of *T. elegans* is distinctive. At a point mid-way on the length of the body there are 23 to 25 rows of scales; there are 23 to 31 rows of scales on the neck, and 19 rows a short distance in front of the anus. There are 179 to 191 ventral scutes and 69 to 76 subcaudals. There are 11 to 14 scales on a line between the supraoculars (large scales bordering the orbit dorsally); and there are usually 8, rarely 9, scales on the upper lip. The nasal plate is divided. All the scales of the body posterior to the head are strongly keeled except those of the outer row (adjoining the ventral scutes). Many of the scales on the head are keeled.

RARE COLOR VARIANT

In 1931 Maki[2] noted that the ground color of the body of some specimens of *T. elegans* was yellowish-brown with a series of pale, dorsal blotches on each side. The lateral spots were faint, and the undersurface was uniformly pale yellow. One such specimen, shown in Figures 5 and 6, was collected at night on a road in the Hoshino area, Ishigaki island by H. L. Keegan and S. Toshioka on 21 November, 1955. Two similar examples were seen in the Maki collection, which is now housed in the Department of Zoology, Kyoto University. Because of the great disparity of appearance between these specimens

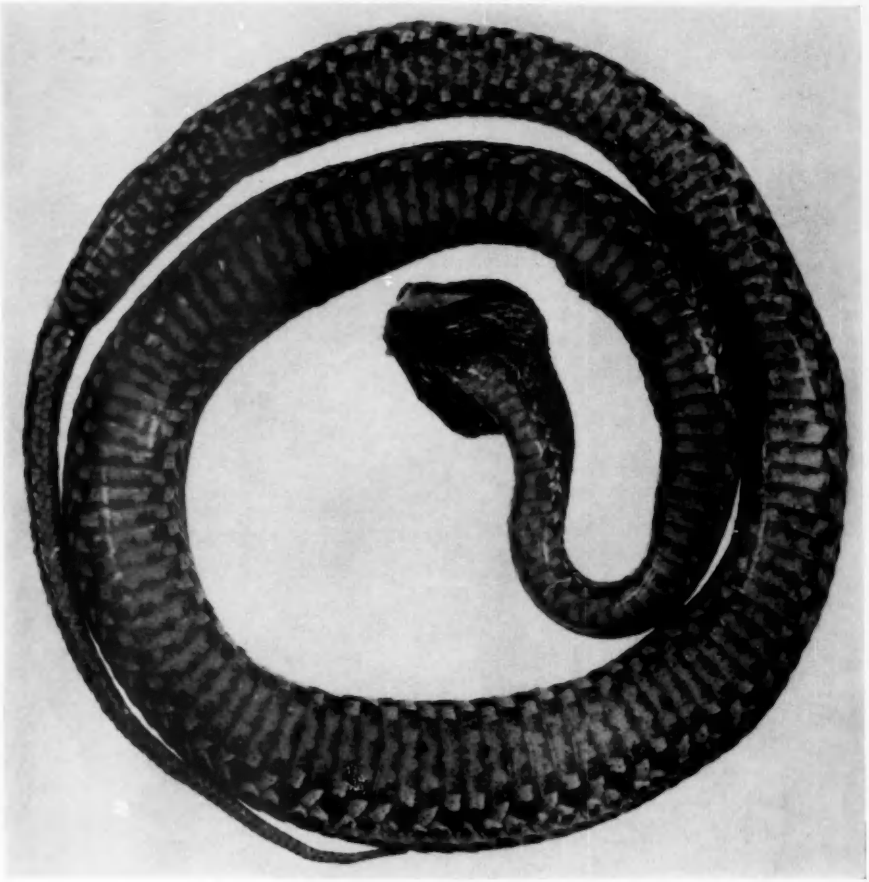

FIG. 4. Ventral view of a specimen of *T. elegans*

and typical *elegans*, a detailed comparison of the two color phases was undertaken. Examples of such extreme variation in color among individuals of a species are difficult to find. It is true that most species of animals exhibit occasional cases of albinism and melanism. The related crotalid snake, *Agkistrodon halys*, the mamushi, shows much variation in color. These color phases of *A. halys*, however, are associated with different geographic regions. In some portions of its range, *halys* is spotted, in others it may be almost solid black above and below, and in still other areas, it may be reddish or rust colored with no discernible pattern.

The following description of the brown "color phase" of *elegans* is based upon the single specimen from Ishigaki. The general appearance is that of a light brown snake with faint dorsal and lateral spots. The color approaches reddish-brown along the mid-dorsal area, and yellowish-brown laterally. The undersurface is clear yellow, with no traces of markings of any kind. The dorsal spots, which are particularly indistinct on the anterior portion of the body, are not edged with black, as shown on typical *elegans*. There appears to be a series of 43 dorsal blotches. A zig-zag arrangement of confluent blotches is faintly visible on the posterior quarter of the body. The end of

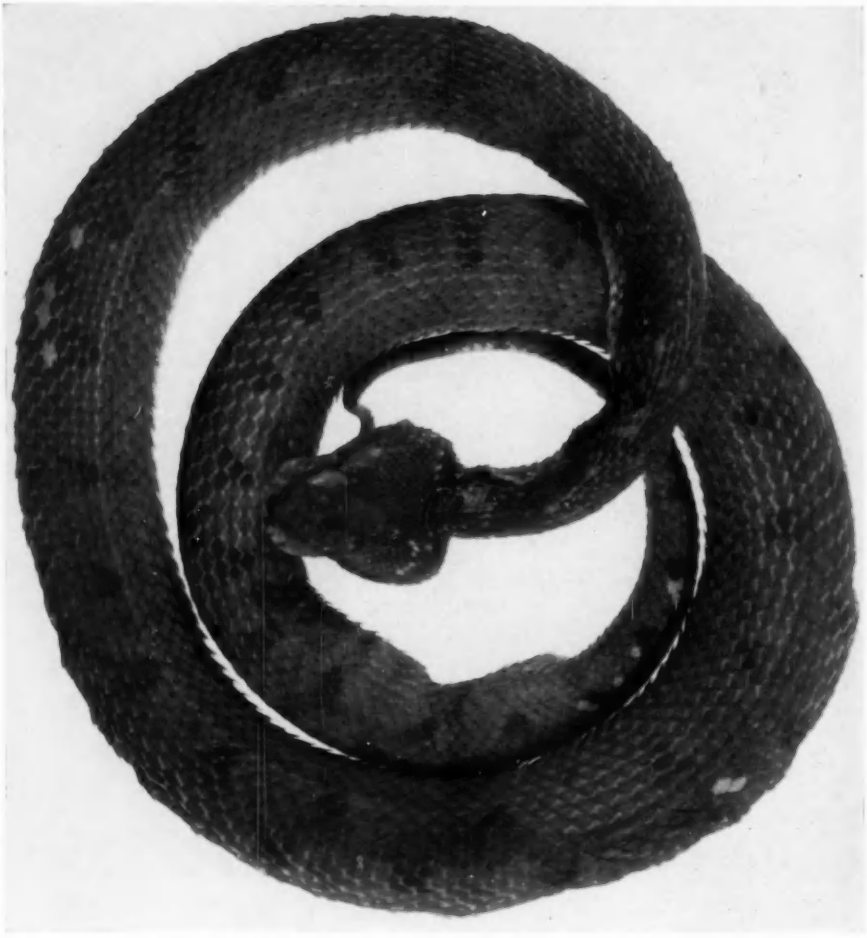

FIG. 5. Dorso-lateral view of a rare color variant of *T. elegans*

the tail is uniform in color. The head and neck are somewhat darker than the remainder of the body of the living specimen. On the undersurface of the head the pale yellow color is tinged with pink. On the dorsal surface of the head are a few blackish flecks. There is only a vague trace of a lateral band extending from the eye through the angle of the jaw. The upper labials are flecked with black. There are no markings on the lower labials.

The general body form is moderate. The tail is gently tapered and pointed at the end; the head is distinct from the neck. The diameter of the neck is 1.4 cm; the diameter of the snake at mid-body is 2.6 cm, and of the body immediately in front of the anus is 1.7 cm. Length of the head is 3.8 cm; its width is 3.0 cm. Total length of the specimen is 96.6 cm, and length of the tail is 14.7 cm.

The anal shield is not divided. One head-length posterior to the head there are 26 rows of scales; at mid-body there are 23 rows of scales, and immediately anterior to the anus there are 20 rows of scales. There are 187 ventral scutes and 71 caudal scutes. The tail terminates in a spine. Arrangement of scales on the head of this speci-

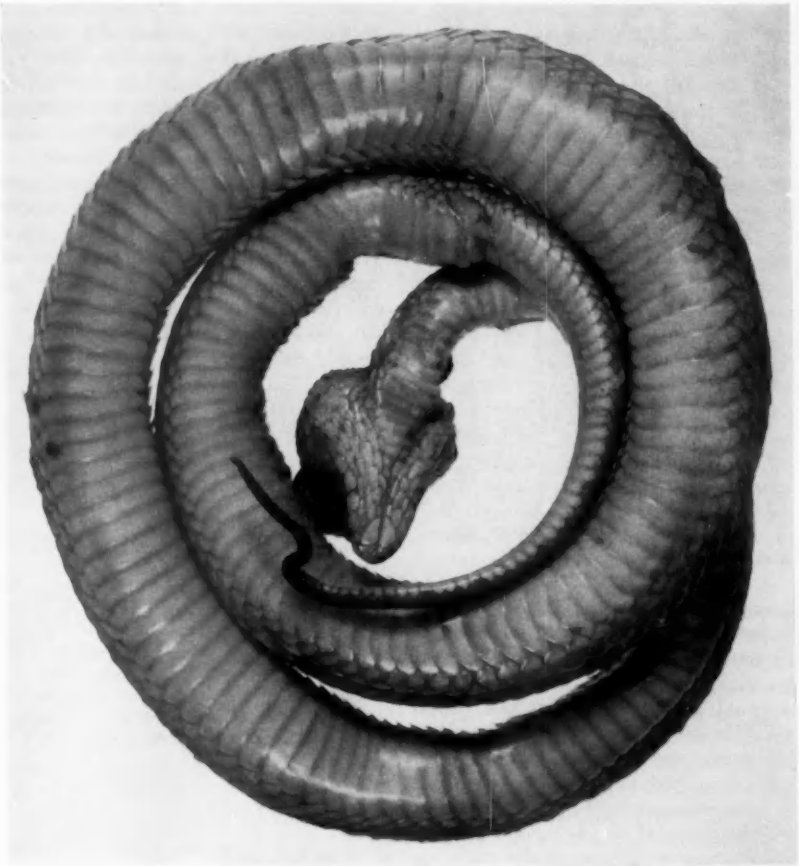

FIG. 6. Ventral view of a rare color variant of *T. elegans*

men is shown in Figures 1 and 7. There are 13 to 14 scales on a line between the supraoculars. There are seven upper labials on either side of the head, and there are ten right and nine left lower labials. The loreal is distinctly longer than wide. The subocular is separated from the subfoveal. Although the humerals are in contact with the upper labials, other aspects of scutellation are essentially as for typical *elegans*.

Data obtained from a study of the two specimens in the Maki collection at Kyoto follow. Collecting data on each of these were incomplete. One of these specimens, a male, is 823 mm in length. On this specimen there are 179 ventral scutes and over 40 subcaudal scutes. The end of the tail is missing. One head-length behind the head there are 29 rows of scales; at mid-body there are 23 scale rows, and immediately in front of the anus there are 19 scale rows. There are eight upper labials on either side of the head and eight right and ten left lower labials. There are 12 scales on a line between the supraoculars. The other specimen, No. 161 in the Maki collection, is a female. This snake is 927 mm in length. It possesses 187 ventral scutes and 62 subcaudal scutes plus a spine. One head-length behind the head there are 29 rows of scales; at mid-body there are 25 rows of scales, and immediately in front of the anus there are 20 scale rows. There are seven upper labials on either side of the head,

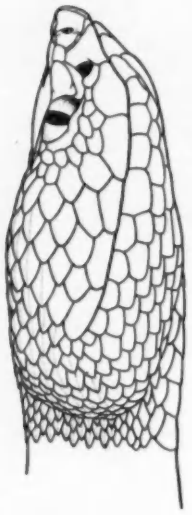

FIG. 7. Lateral view of the head of *T. elegans*, the Sakishima habu.

and ten lower labials on either side. There are 12 to 13 scales on a line between the supraoculars.

These data reveal no clear-cut differences, other than color, between the three brown habu examined and specimens with typical *elegans* markings. The loreal shield *may* be relatively longer on each of the brown specimens, and the apical pit of the body scales of typical *elegans* may be more pronounced. On brown specimens examined, the humeral shields which touch the upper labials were relatively smaller than the others and the fourth upper labial was at least as large as the third. The only other possible differences in scutellation are in the anterior chin shields, and in the supraoculars. The anterior chin shields of brown specimens were relatively smaller than those of typical *elegans*, and the supraoculars were not quite as wide as described by Maki for *elegans*. Since all other aspects of scutellation of both forms are alike, it is highly probable that the slight apparent differences noted are matters of individual variation. Hemipenes of males have not been compared.

VENOM POTENCY AND NEUTRALIZATION BY ANTIVENIN

At present there are no effective antivenins for treatment of bites by the Sakishima habu. This was shown in 1956 by neutralization tests performed at Wyeth Laboratories in the United States, and at the University of Tokyo. Since the Wyeth product, Antivenin Crotalidae Polyvalent (North and South American Antisnakebite Serum), was relatively potent in neutralizing venoms of some related Asian pit vipers, these results were surprising. Information obtained during the series of tests at Wyeth Laboratories is summarized in Table 1. Venom samples used in these tests were from a variety of sources. Venoms of *T. elegans* and *T. flavoviridis* were collected and lyophilized at the 406th Medical General Laboratory. A sample of *flavoviridis* venom was also obtained from the University of Tokyo. Freshly collected venom of *T. wagleri* was sent to the 406th Medical General Laboratory from the Institute for Medical Research, Kuala Lumpur, Malaya. Other venoms listed in Table 1 were forwarded directly to Wyeth Laboratories from the Taiwan Serum Vaccine Laboratory, Taipei, Taiwan.

TABLE 1

*Summary of results of neutralization tests performed to determine potency of Antivenin Crotalidae Polyvalent (North and South American Antisnakebite Serum) against venoms of some Asian pit vipers**

Venom	LD 50/mg	Antivenin Lot No	LD_{50} of venom neutralized per ml of antivenin
Agkistrodon acutus	12.0	039	<2.0
Trimeresurus gramineus	50.0	039	72.0
T. mucrosquamatus	33.5	039	76.0
T. elegans	11.6	039	<2.0
T. wagleri	70.0	024	<6.0
T. wagleri	70.0	040	<6.0
T. flavoviridis	17.8	024	20.6
T. flavoviridis	17.2	024	25.4
T. flavoviridis	16.0	040	18.0
T. flavoviridis	18.6	040	22.0
T. flavoviridis	16.0	040	17.8
T. flavoviridis†	10.0	02411	27.0
T. flavoviridis	10.0	02411	28.0
T. flavoviridis	10.0	03901	15.4
T. flavoviridis	10.0	03901	15.0

* In these potency and neutralization tests intravenous venom and antivenin injections were given to white mice. Venom potency is expressed in mouse LD 50/mg. Antivenin potency is expressed by numbers of mouse LD_{50} of venom neutralized by one ml of antivenin.

† Venom from University of Tokyo.

Results similar to those obtained at Wyeth Laboratories were obtained in neutralization tests at Tokyo University. These tests, which were conducted by Dr. S. Miyazaki, utilized *T. elegans* venom from the 406th Medical General Laboratory and habu antivenin produced at Tokyo University. This product was prepared by immunizing horses with the venom of *T. f. flavoviridis*. Dr. Miyazaki reported that there was no appreciable neutralization of *T. elegans* venom with the antivenin used in the tests.

PART II

NOTE

Kozen Yoshino is a practicing physician in Shika, Ishigaki island. Because of his experience in treatment of snake bite on that island, he was asked to write his observations for publication. His manuscript, which was written in Japanese, was translated by Dr. Joe Niiya of the 406th Medical General Laboratory. Although some changes were made in arrangement of sections of the manuscript for this paper, all factual material which follows is entirely the work of Dr. Yoshino. As far as the author was able to determine, these are the first published clinical observations on symptoms caused by the bite of *T. elegans*, and on treatment of bites by snakes of that species.

INTRODUCTION

Prior to World War II the writer treated many patients who had been bitten by specimens of *Trimeresurus elegans* on islands of Yaeyama-retto. Clinical records of these cases were lost during the war and observations on this problem were resumed in December, 1950. Between this time and December, 1955, a total of 24 cases was seen. Records were kept not only on clinical matters, but also on circumstances in which snake bites occurred. These observations are summarized in the following, brief report.

SNAKE BITE ON ISLANDS OF YAEYAMA-RETTO

Cases of snake bite among man and domestic animals due to the Sakishima habu, *T. elegans*, have long been known from several of the islands of Yaeyama-retto. These include Ishigaki-jima, Iriomote-jima, Kohama-jima, Juro-jima, and Taketomi-jima. The Sakishima habu is not known to occur on Yonakuni-jima, Hateruma-jima, Nakanokami-jima, Senkahu-jima, or Hatoma-jima.

The Sakishima habu is found in a variety of habitats. It occurs in lowlands and in mountainous areas, but is most often seen among stone walls, old houses, cemeteries, and in bamboo groves and other vegetation. It is rarely seen during the daytime, but is very active at night. *T. elegans* feeds on mice, frogs, and small birds and their eggs. Female snakes lay their eggs during the summer. The writer has seen a nest containing several eggs which were oblong and white in color. Although this snake is of a terrifying appearance, it does not seem to be as aggressive as the Okinawan habu. It very seldom takes the initiative in attacking man or large animals. It is true that some specimens may strike at the legs of men who walk near them, and they have been known to climb trees and strike from that position. Some of the local people believe that this snake can spring about 3 feet, but the writer has never observed this. Another local belief is that the snake's breathing can be heard if the snake is nearby. This too, has not been confirmed by the writer.

Most of the cases of snake bite reported in this paper were seen during the summer and autumn months. As might be expected from our knowledge of the habits of the Sakishima habu, more persons were bitten at night than during the daytime. Incidence of snake bite was greatest among those who worked in the fields. Of the 24 cases reported here, 20 were males, and only three of these were under 10 years of age. Five persons were bitten in their own yards, four were bitten while working in fields planted with potatoes and soya beans, four persons were bitten while walking on the grassy ridges of rice fields, and four men were bitten while working in wooded areas. Three persons were bitten while walking along roads at night, and others were bitten near old graves, or in the plains.

Fifteen of the 24 patients in this series received bites on the legs, eight received bites on the hands and one person was bitten on the back of the head by a snake which struck from a tree.

Symptoms

There was considerable variation in severity of symptoms among patients seen by the writer. This was probably due to differences in quantity of venom injected, in location of the bite, and in size and physical condition of the patient. Natural appearance of the bitten area following the

bite was not observed in all cases, as some persons applied first aid measures before reporting for treatment. These measures consisted of incising the bitten area, or burning the area surrounding the fang puncture with matches. A towel or other binding material was usually applied as a tourniquet.

In persons who had not received such first aid, 2 to 4 hemorrhagic dots marked the site of the bite. It is believed that these were fang marks, but in cases where 4 dots were present, it was not determined whether the 2 extra dots were caused by supplementary fangs or a second bite. In all but 2 of the 24 cases in this series, only local symptoms were observed. In all patients the area around the fang marks was swollen and painful when touched. Some patients complained of spontaneous pain, or were cyanotic. In most cases where severe spontaneous pain or cyanosis was present, the bitten area became necrotic. In one such patient, bitten on the hand, necrosis of tendon, muscle and bone occurred, and the thumb was lost. In another instance necrosis, following a bite on the ventral aspect of the right middle finger, was so severe that resulting tendon and muscle destruction caused a permanent curvature of the finger.

One of the 2 patients who developed general symptoms was a 6-year-old boy, who was bitten on the right thumb by a habu about 3 feet in length. Hemorrhagic marks were found at the bitten site and cyanosis followed initial swelling and pain. Three hours after being bitten the patient developed accelerated respiration, the pulse was weak, and the finger nails were cyanotic. The patient became comatose, and was considered to be critically ill. It was thought that he was going to die, but on the second day he regained consciousness, and his respiration and pulse became normal. Except for local necrosis and loss of his thumb, the patient made a full recovery. Treatment in this case included incision of the bitten area, and administration of habu antivenin, Ringer's solution, and heart stimulant. The other patient who developed general symptoms was a 56-year-old male. This man was bitten on the ventral aspect of the right middle finger by a habu 3 feet in length. The physician made an incision immediately, and administered habu antivenin. There was hemorrhage at the bite site, along with swelling, spontaneous pain, and cyanosis. The patient was never in critical condition, but complained of insomnia due to the severe pain. This is the patient who suffered a permanent curvature of the finger.

Treatment

In each case seen by the writer, the bitten area was incised and cleaned as soon as possible. Following the advice of earlier workers, the writer applied dressings with a 5% solution of $KMnO_4$ in five cases. Since these dressings were apparently without therapeutic effect, they were not employed in treatment of the following 19 cases in this series.

Antivenin produced in Tokyo and recommended by the Government of the Ryukyus was used in treatment of 17 of the 24 cases. As all patients recovered, it was difficult to judge the effectiveness of the antivenin in this small series of cases. Recently, the Government of the Ryukyus has been recommending an antivenin manufactured by the Wyeth Laboratories in the United States. The author has never used this product, and has no information as to its effectiveness in the treatment of snake bite.

Supportive treatment included use of vita-camphor, cardiac stimulants, Ringer's solution, and dextrose injections. Tissue damage occurred in some patients who had left tight bandages in place for long periods of time. There was also some local tissue damage in patients who had burned the bitten area as a first-aid measure.

Prognosis for patients bitten by the Sakishima habu is good. The writter has seen only one fatal case of snake bite due to *T. elegans*. This single case was that of a 54-year-old male, who was bitten on the median aspect of the left ankle on 25 March, 1926. An incision was made, and antivenin, Ringer's solution, and cardiac stimulants were administered. The bitten area was marked with hemorrhagic spots, and there were considerable swelling, pain and cyanosis. Several hours after the bite occurred, the patient experienced difficulty in breathing. Chest pains were felt, the pulse became fast and weak, and the patient's nails became cyanotic. The patient's condition became critical, and he expired 24 hours after being bitten.

SUMMARY

The Sakishima habu, *Trimeresurus elegans* (Gray, 1849) has been reported only on islands of Yaeyama-retto, Sakishima-gunto, about 240 miles south of Okinawa. Color and scutellation serve to distinguish this species from other pit-

vipers of the Ryukyu islands and Taiwan. Reddish-brown snakes, differing from typical *elegans* only in coloration, are rarely taken. *T. elegans* is smaller than the Okinawan habu, and adult specimens are usually between 3 and 4 feet in length. On the islands where it occurs, *T. elegans* is found in several types of terrain, and is active mainly at night.

Venom of *T. elegans* was not neutralized in therapeutically significant amounts by an antivenin produced at Tokyo University, or by a polyvalent antivenin produced by Wyeth Laboratories. The latter product, Antivenin Crotalidae Polyvalent (North and South American Antisnakebite Serum), was highly effective in neutralizing venoms of some Asian species of the genera *Trimeresurus* and *Agkistrodon*, but almost without effect against venoms of other snakes of these genera.

Clinical observations reported were based on a series of 24 cases of snake bite due to *T. elegans*, which occurred between December, 1950, and December, 1955, on islands of Yaeyama-retto. Most of these bites had been inflicted at night, and were seen during summer and autumn months. Most of the bites were located on the legs and hands. Symptoms included hemorrhagic spots at fang punctures, pain, swelling and cyanosis. Localized necrosis was severe in some patients. Only two patients of the 24 in the series developed general symptoms.

Treatment included incision and cleaning of the bitten area; administration of antivenin produced in Tokyo; and supportive treatment, including use of vita-camphor, cardiac stimulants, Ringer's solution, and dextrose injections. Tissue damage occurred in some patients who had applied tourniquets and had allowed them to remain in place for long periods of time. There was also some local tissue damage in patients who had burned the bitten areas as a first-aid measure. Dressings with 5% solution of $KMnO_4$ were applied in five cases, but were discontinued when they proved to be without therapeutic effect.

Only one fatal case was seen. This patient, an adult male, developed the usual symptoms, and several hours after the bite was inflicted experienced difficulty in breathing. Chest pains were felt; the pulse became fast and weak; the nails became cyanotic, and the patient expired 24 hours after being bitten.

ACKNOWLEDGMENTS

Many persons assisted the writers in the field and laboratory. 406th Medical General Laboratory personnel who aided in this work are: Mr. Seiichi Toshioka, Advisor, Dept. of Entomology; Miss Yoshiko Yoshida, Advisor, Dept. of Entomology; Miss Kinuyo Kamei, Entomological Artist, Taxonomic Section, Dept. of Entomology; SFC Paul J. Weber, Dept. of Medical Illustration; and Dr. Joe Niiya, Interpreter. Antivenin potency studies were made possible through the courtesy of Mrs. Eleanor E. Buckley and Dr. B. R. Criley of Wyeth Laboratories, and Dr. S. Miyazaki of Tokyo University. Samples of the venom of *T. wagleri*, a Malayan pit viper, were collected at the Institute for Medical Research, Kuala Lumpur, Malaya and forwarded through the courtesy of Mr. John Waters and Lieutenant Colonel Robert Traub, MSC, USA.

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FIELD OBSERVATIONS ON THE BITING HABITS OF *CULEX TARSALIS* AT MITCHELL, NEBRASKA, AND LOGAN, UTAH

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Since 1941, *Culex tarsalis* Coquillett has been recognized as a primary vector of the viruses of western and St. Louis encephalitis in the western half of the United States (Hammon *et al.*, 1941, 1945). It is paradoxical, however, that the literature contains widely conflicting views concerning the blood-feeding habits of this important mosquito. A number of workers have implied that *C. tarsalis* feeds on man only occasionally (Freeborn, 1926; Riley and Chalgren, 1939; Rozeboom, 1942; Rees, 1943; Tate and Gates, 1944; Brookman, 1950; Jenkins, 1950). Several other workers, however, have stated that this species is a painful or fierce biter and readily enters houses in search of blood (Hearle, 1926; Mail, 1934; Matheson, 1944; Ross, 1947). It appears that most of the generalizations relative to the biting habits of *C. tarsalis* have been based on casual impressions rather than upon actual field studies.

In 1953 and 1954, when the writer was stationed at Mitchell (Scotts Bluff County) Nebraska, and Logan (Cache County) Utah, respectively, opportunity was afforded to study the biting habits of this species in two widely separated irrigated areas. Mitchell (population 2,101) is situated within the Great Plains area at 41° 56′ north latitude and 103° 49′ longitude; its elevation is approximately 3,800 feet. Logan (population 16,832) is located in an inter-mountain area at 41° 41′ latitude and 111° 49′ longitude; on the valley floor, the elevation is about 4,500 feet.

In both areas, systematic biting studies were conducted for an entire season. The main purpose of these studies was to determine (1) the extent to which *C. tarsalis* bites man under field conditions, (2) the time of day of peak biting activity, and (3) the seasonal trend of feeding activities. A lesser objective was to obtain data on the animal-biting (zoophilic and ornithophilic) characteristics of *C. tarsalis*.

METHODS

Mitchell, Nebraska. Two collecting sites were chosen for detailed observations, one at the writer's residence in the center of Mitchell, and one at a farmstead approximately 1 mile north of town. Both situations were well protected by trees, shrubbery, etc. Domestic animals and fowl present in the immediate vicinity of the sites included two chickens on the urban premises and about a dozen sheep at the farm. Wild birds were prevalent at both sites; the use of an elm tree in the writer's front yard as a nocturnal roosting site for robins was particularly noticeable.

In order to have comparative baseline information on mosquito prevalence and activity, a New Jersey light trap, equipped with a 25-watt bulb, was operated nightly at each site from approximately sunset to sunrise. The traps were situated out of view of the biting stations.

The following procedure was used in obtaining the routine man-biting records. Collections were started at sunset and continued for 2 hours; during this period, eight consecutive 15-minute collections were made. While the operator was seated on a stool or chair under a tree, collections were made from the exposed legs by the use of a chloroform killing tube (8 inch x 1 inch) equipped with a paper funnel in its mouth (Fig. 1). An attempt was made to collect the mosquitoes as soon as they lit to feed rather than to wait for them to bite. For the collections made after dark, a flashlight, with the lens shaded by means of red cellophane, was used. At the end of the 2-hour collecting period, the mosquitoes were transferred from the killing tubes to pill boxes. Air temperature readings were taken and recorded at the midpoint of each quarter hour.

In order to obviate possible individual differences in attractiveness to mosquitoes, 5 collectors participated in the biting studies (majority of collections were made by three: G.A.M., L.R.E., and L.D.B.). They rotated between the two areas on different nights. An average of four collections per week was made at each site during the active mosquito season, June 8 to September 1, 1953. During the period September 2 to October 8, eight collections were made at the urban location and 24 at the farmstead.

FIG. 1. Technique used in collecting mosquitoes after dark at rural site, Mitchell, Nebr.

Incidental observations on the animal-biting habits of *C. tarsalis* were obtained by hand collections of mosquitoes from a tethered cow and horse; by operation of a large stable bait trap with a sheep and calf as an attractant; and by a small stable trap with one or two chickens as bait.

Logan, Utah. The two collecting sites were the writer's back yard and a neighbor's adjoining property in southwest Logan, and a farmstead located 1.7 miles west of the city. Although a few wild birds were present at the urban site, there was no concentration of individuals such as was found at Mitchell. On the farm, there was an abundance of dairy cattle, sheep, and chickens.

The method of study was similar to that described above, including the operation of a light trap in each area. The principal differences were that only the writer participated in the study, and some data on light intensity during the twilight period were obtained by the use of an illumination meter (Weston photometer), which was sensitive to 0.1 footcandle. The number of collections averaged two per week in town and three in the country. The period of observation was chiefly from June 3 to August 31, 1954. Eight preliminary observations (1-hour evening collections) were made at the farm from May 12 to 29. Unfortunately, due to the occurrence of an outbreak of encephalitis in Hidalgo County, Texas, the writer was unable to make biting collections from September 1 to 16. The last collections were made during standard week number 38, from September 17 to 23.

OBSERVATIONS

Relative prevalence of C. tarsalis. Table 1 shows the relative abundance of female mosquitoes of the major species taken during the manbiting collections and the all-night light-trap collections. At Mitchell, Nebr., *C. tarsalis* ranked first in biting at the rural site and second to *Aedes vexans* in town. At Logan, Utah, it was the most numerous biter in the city and second at the farm. The two most abundant species in the two areas, as measured by light trap catches, also were *C. tarsalis* and *A. vexans.*

Table 2 shows that *C. tarsalis* was attracted readily to various farm animals and domestic fowl in the Mitchell area. It will be noted that this mosquito was the predominant species feeding on a cow, a calf, a sheep, and the chickens; in two tests with a horse as an attractant it ranked third, being exceeded by *A. vexans* and *Aedes dorsalis.*

It is noteworthy that *C. tarsalis* made no particular effort to enter the writer's residence at Mitchell, even though it was exceedingly common on the premises. Of 34 female mosquitoes collected from inside the house during the period June 15 to September 27, 1953, not a single specimen of *C. tarsalis* was found (26 specimens were *Aedes triseriatus*). This observation indicates that owing to the availability of a good

TABLE 1

Relative abundance of female mosquitoes taken in man-biting collections and light-trap collections at an urban and rural site, Mitchell, Nebraska, 1953, and Logan, Utah, 1954

Species*	Percentage of total collection							
	Biting (evening)				Light trap (all-night)			
	Mitchell, Nebr.		Logan, Utah		Mitchell, Nebr.		Logan, Utah	
	Urban	Rural	Urban	Rural	Urban	Rural	Urban	Rural
Aedes vexans	67	36	41	34	45	51	17	43
Culex tarsalis	24	43	50	25	20	27	52	24
Aedes dorsalis	5	11	1	1	31	13	1	2
Mansonia perturbans	<1	1	1	24	<1	<1	1	8
Culiseta inornata	<1	2	<1	2	<1	8	5	12
Anopheles freeborni	0	0	3	7	0	0	8	3
Culex erythrothorax	0	0	2	1	0	0	14	4
Aedes nigromaculis	2	2	1	4	2	<1	<1	3
Aedes triseriatus	1	2	0	0	<1	<1	0	0
Total number females	4,024	8,032	1,330	10,779	3,296	15,507	471	12,677
Number of collections	63	71	25	51	63	71	25	51

* Minor species excluded.

TABLE 2

Percentage of three main species of mosquitoes taken from livestock and domestic fowl, June 17–Aug. 29, 1953, Mitchell, Nebr.

Host	Collecting method*	Total mosquitoes	*Culex tarsalis*	*Aedes vexans*	*Aedes dorsalis*
Chickens	bait trap	2,101	87	11	1†
Sheep	bait trap	60	86	5	6
Cow	hand	179	47	27	16
Calf	bait trap	613	41	30	22
Horse	hand	1,984	8	73	11

* Hand collections: 2-hour periods following sunset; bait traps operated all-night.

† Percentages do not total 100 since minor species are excluded.

source of food from a robin roost, chickens, and other birds on the premises, there was little need for *C. tarsalis* to enter a dwelling in search of a blood meal. Blood-engorged specimens of *C. tarsalis* were frequently observed resting on the front porch and in an ornamental juniper shrub adjacent thereto. Precipitin tests run on 446 samples of these engorged mosquitoes showed that 431 had fed on passerine birds and three on cattle; 12 showed no reaction.

In contrast to the Mitchell findings, 91 of 99 female mosquitoes collected inside the writer's house at Logan (April 19 to September 27, 1954) were *C. tarsalis*. Thirty of these specimens were taken during one 4-day period, July 27 to 30. Several of these mosquitoes attempted to bite during the evening hours, in both lighted and dark rooms.

Peak period of biting. Figure 2 demonstrates clearly that *C. tarsalis* is a nocturnal species in its feeding activities. Only 5.8 and 7.7 per cent of the total 2-hour collections were taken during the first 30 minutes after sunset at the Mitchell and Logan farms, respectively. The peak of biting activity was found to start 30 minutes after sunset and to continue for 1 hour (periods 3 through 6). In both areas, the largest catches were made during the fourth 15-minute period. This is in contrast to the peak activity for *Anopheles freeborni* (second period), *A. vexans* (third period), and *Mansonia perturbans* (fifth period) (Beadle, 1955). The time of day for the maximum 1-hour period of biting activity for *C. tarsalis* ranged from 7:15 to 8:45 p.m. (July 25 to August 26) at Mitchell and from 8:00 to 9:30 p.m. (July 11 to August 12) at Logan.

The beginning of the peak period (fourth) commenced from about 10 to 16 minutes after the end of civil twilight (sun less than 6° below

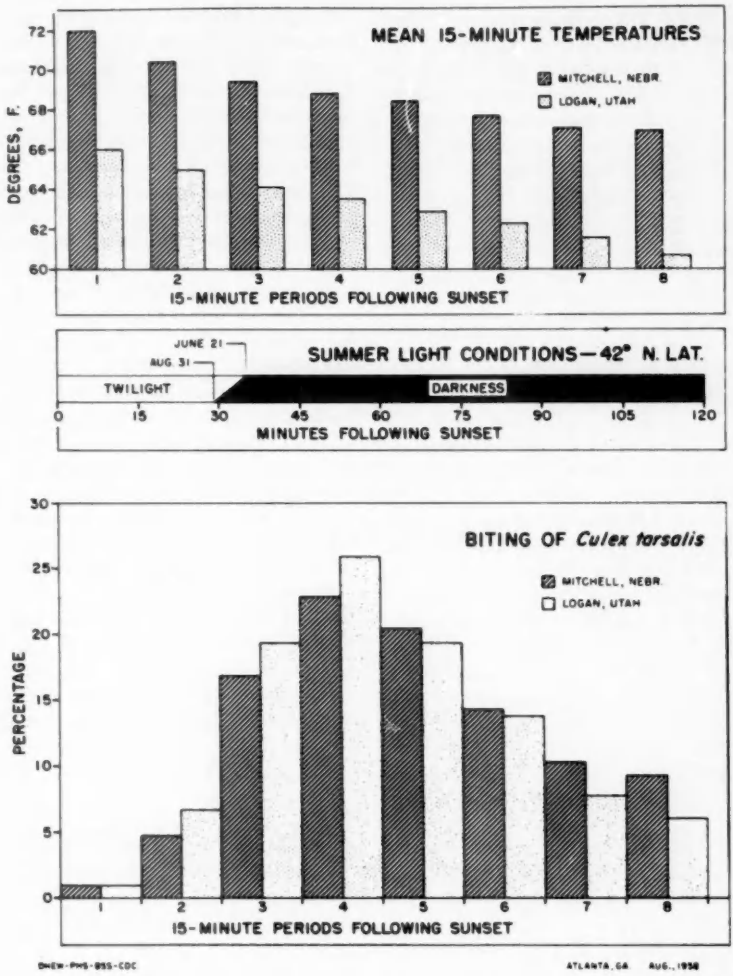

FIG. 2. Time of biting of *Culex tarsalis* in relation to sunset and the daily cycles of light and temperature. Data are based on 47 collections (3,487 *C. tarsalis*) at Mitchell, Nebr., June 13–Sept. 1, 1953, and 40 collections (2,722 *C. tarsalis*) at Logan, Utah, June 3–Aug. 31, 1954.

the horizon). The duration of twilight at 42° N. Lat. is 35 minutes on June 21 and 29 minutes on August 31 (Eckert and Clemence, 1945) (Fig. 2).

Photometer readings at sunset in the Logan area showed a mean of 5.3 foot-candles at the farm and 7.5 foot-candles in town (readings under shade of tree). During intervals averaging approximately 20 and 24 minutes, respectively, the light intensity diminished to 0.2 foot-candle; this reading usually corresponded with the beginning of the first "wave" of crepuscular mosquitoes.

Mean temperature readings for the eight 15-minute periods varied from 72°F. to 66.8° at Mitchell and 66.0° to 60.6° at Logan (Fig. 2). The mean temperatures for period 4 were 68.8° and 63.5°, respectively. In an analysis of the season's largest 15-minute catches of five or more

C. tarsalis for any period, it was found that the temperatures at Mitchell ranged from 58° to 78°, with a mean of 67.9°; at Logan from 56° to 78°, with a mean of 65.4°. The lowest temperature at which *C. tarsalis* was taken was 47° (July 22, 1954, Logan).

TABLE 3

Hourly catches of Culex tarsalis *during all-night biting collections July 24–25, 1953, Mitchell, Nebr.*

Hours	Temp. (°F.)	*Culex tarsalis*
P.M.		
7–8*	73.5	30
8–9	73.7	78
9–10	74.7	83
10–11	71.5	77
11–12	68.0	51
A.M.		
12–1	64.5	50
1–2	66.2	13
2–3	64.7	13
3–4	63.2	7
4–5*	60.0	2
Total		404

* Sunset 7:20; sunrise 4:40.

It is believed that the peak of biting activity for *C. tarsalis* is governed principally by light intensity and temperature. Maximum biting does not begin until the period of twilight has ended, and reduction in activity after period 4 is undoubtedly correlated with the normal decline in temperatures as night advances. During nights with more or less constant temperatures or with rising temperatures beyond the 2-hour post-sunset period, the period of maximum biting may be greatly prolonged. This is illustrated in Table 3, which summarizes hourly catches of *C. tarsalis* for a 10-hour period from sunset on July 24 to sunrise on July 25, 1953. It will be noted that biting rates were well sustained until 1:00 a.m.

Seasonal feeding activities. The average evening biting and nightly light-trap catch per week for the two urban and rural sites is shown graphically in Figure 3. In the Mitchell area, the greatest number of *C. tarsalis* attracted to the human bait occurred between July 25 and August 26; in the Logan area, between July 11 and August 12. These data appear to be related to the commencement of *C. tarsalis* breeding in the spring. The first captures of males in light traps were on May 16 and May 28 at Logan and Mitchell, respectively.

In biting intensity, the mean numbers of *C. tarsalis* per man/hour of exposure (periods 3

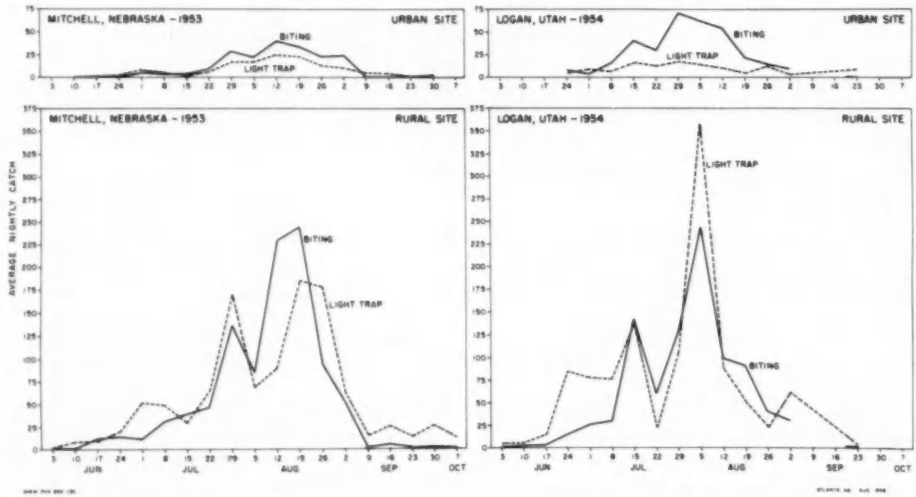

FIG. 3. A comparison of 2-hour evening biting collections and all-night light-trap catches of female *Culex tarsalis* at an urban and rural site, Mitchell, Nebr., 1953, and Logan, Utah, 1954.

through 6) for the months of July and August were as follows: Mitchell: urban, 13, rural 79; Logan: urban, 26, rural 67. Maximum 2-hour biting collections of this species for the two areas were: Mitchell: urban 75 (Aug. 12), rural 364 (Aug. 12); Logan: urban 87 (July 29), rural 287 (Aug. 2).

One of the most striking observations during these studies was the drastic decline of blood-feeding activities by *C. tarsalis* following September 1 at Mitchell (Fig. 3). This sudden change in behavior seemed to be triggered by a sharp drop in temperature (30°F drop in 2 hours) that occurred the afternoon of September 2; at sunset the temperature was only 53°F and by next morning it had dropped to 43°; the lowest temperature of the summer up to that date. Of 538 mosquitoes taken during 32 biting collections from September 2 to October 8, only 45 were *C. tarsalis* (in contrast, 51 *C. tarsalis* were taken in one biting collection on September 1).

Although biting collections at Logan were not made from September 1 to 16, there is strong evidence that this same phenomenon occurred, and this has been confirmed by observations in subsequent years. On August 31, a total of 26 *C. tarsalis* attempted to bite; in five collections from September 17 to 21, only one specimen of this species was taken. In routine collections of *C. tarsalis* from shelters during the month of September, the last blood-engorged specimens were taken on September 16. This observation indicates that *C. tarsalis* exhibits a general, late-season (prehibernation) decline in blood-feeding and that it is not limited only to humans.

DISCUSSION

Although light-trap and biting collections give indices for two different types of mosquito activity, results of studies reported herein demonstrate that both sampling methods were quite consistent in measuring *C. tarsalis* populations throughout the summer months (Fig. 3). The main exception was the urban trap in Logan. The poor showing of this trap undoubtedly reflects the competition with lights of the city. If this hypothesis is correct, it should be possible to increase the efficiency of traps operated in a city by increasing the wattage of the light bulb.

The results of the biting studies in the two widely separated areas indicate that *C. tarsalis* will feed readily on a wide range of hosts, including wild birds, chickens, cattle, sheep, and man. It appears that *availability of host* is the main criterion for this mosquito with such catholic tastes.

With reference to statements in the literature on the frequency with which *C. tarsalis* enters human dwellings in seeking blood meals, our studies suggest that if a natural food supply is available in the immediate vicinity, no particular effort is made to enter houses; if absent, *C. tarsalis* may enter houses readily. Although *C. tarsalis* was found to bite aggressively on several occasions, it was not considered a "painful" biter.

In both areas, a marked decline in blood-feeding activities was observed early in September. Studies on the hibernation of *C. tarsalis* in the Greeley, Colorado area indicate that females from late summer and fall broods take plant juice and enter a diapause with respect to blood feeding to develop fat bodies (Bennington, 1958). Recent laboratory studies in California suggest that blood feeding in autumn is prejudicial to survival of *C. tarsalis* through the winter (Reeves *et al.*, 1958). The decline in blood-feeding in the fall might decrease the potentialities of *C. tarsalis* as an overwintering reservoir for encephalitis viruses.

In view of the fact that peak biting was found to occur during the first hour of full darkness, this time of evening probably represents the greatest hazard from the standpoint of encephalitis transmission. In the event of an outbreak of encephalitis, consideration might well be given to establishing a curfew at this particular time as an emergency measure. This same hour would seem to be an excellent period for intensification of control efforts, such as space sprays, directed against adult *C. tarsalis* populations.

SUMMARY AND CONCLUSIONS

Systematic man-biting collections of mosquitoes were made at an urban and a rural site at Mitchell, Nebraska, in 1953, and at Logan, Utah, in 1954. *Culex tarsalis* ranked first in biting at the rural site in Mitchell (43% of 8,032 mosquitoes) and at the urban site in Logan (50% of 1,330). It ranked second to *Aedes vexans* at the urban site in Mitchell (24% of 4,024) and at the farmstead in Logan (25% of 10,779).

C. tarsalis was found to have a strong affinity for man, also for other hosts such as wild birds, chickens, and cattle, during the period from June

until early September. Availability of host seemed to be the main criterion. With the advent of cool weather in the fall, *C. tarsalis* was found to feed reluctantly on man and other vertebrate hosts.

In both areas (42° N. Lat.), the normal peak activity started 30 minutes after sunset and lasted for 1 hour. The largest catches were made during the fourth 15-minute period following sunset.

Large catches of *C. tarsalis* were made at temperatures ranging from 58°F to 78° at Mitchell and from 56° to 78° at Logan. The mean temperature for the fourth quarter-hour period following sunset was 68.8° at Mitchell and 63.5° at Logan.

In Mitchell, the largest catches of *C. tarsalis* were made between July 25 and August 26; in Logan between July 11 and August 12. In biting intensity, the mean numbers of *C. tarsalis* per man/hour of exposure (quarter-hour periods 3 through 6) for the months of July and August varied from 13 to 79 at Mitchell, and 26 to 67 at Logan.

These studies suggest that avoidance of human exposure to mosquito bites during the first hour of summer darkness might serve as a practical prophylactic measure from the standpoint of encephalitis transmission. This same hour also would seem to be an excellent period for intensification of control efforts directed against adult *C. tarsalis* populations.

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WHEN BUBONIC PLAGUE CAME TO CHINATOWN

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For fifteen years Chick Gin, a subject of the Emperor of China, toiled unnoticed among his countrymen in California. Then, on the Monday morning of March fifth, 1900, he emerged to become first on stage in a drama whose cast was to include the President of the United States, several governors, and other notables. The audience was to be critical and vociferous, but Chick Gin spoke no lines. He was not even a walk-on. For, when the curtain rose, he lay dead in a cellar at 1001 Dupont Street, in San Francisco's Chinatown. What he died of, and what provoked the commotion that followed, was bubonic plague. Chick Gin was the first recognized case originating in North America.

A city physician, F. P. Wilson, kindled suspicion. The health department's bacteriologist, Dr. Wilfred H. Kellogg, checked preparations of lymph nodes under his microscope, then hurried to Angel Island to give his material to the chief quarantine officer, Surgeon Joseph J. Kinyoun of the United States Marine Hospital Service, who injected it into rats, rabbits, and a monkey. Meanwhile, Health Officer A. P. O'Brien isolated Chinatown within a police cordon and began a house-to-house search for more cases.

On Wednesday, newspapers reported Chick Gin's death, and simultaneously attacked the health officials: "That there are in the world men so lost to all sense of humanity as for personal ends to bring upon their community the horrors of a plague scare is all too true . . . . A miserable Chinese who had died of a common form of a blood disease is now alleged by politicians whom Mayor Phelan has foisted upon the city to have died possibly of bubonic plague. There is not the slightest evidence that such is the case."

In Washington, the Surgeon General of the Marine Hospital Service, Walter Wyman, read the news dispatches, and immediately wired his ranking officer in San Francisco, Surgeon James M. Gassaway. In essence, Dr. Wyman wanted to know: "What's going on out there?"

For a day or so, Gassaway could have answered, "Not much." When the search of Chinatown had revealed no more plague by Friday, the health officer raised the quarantine. Newspapers chorused: "Plague fake is exploded . . . health board forced to abandon its bubonic bluff."

But out on Angel Island, Kinyoun's animals were sickening. On Sunday, health officials gathered in his laboratory. Assistant Surgeons Leslie L. Lumsden and John W. Kerr, sensing the pressure on their chief, suggested repetition of his tests before he committed himself, but Kinyoun said, "No. This can be nothing but plague. I shall announce it."

Dr. O'Brien reapplied the quarantine. Thirty inspectors combed the 12-block area harboring 25,000 Chinese. Newspapers ridiculed the "fruitless search for germs in Chinatown," asserting that "the bubonic scare has collapsed." They accused the health officer of being "still after the city's money."

The health department certainly needed money. O'Brien and many of his staff served without pay. The "excess amount of violent press criticism" threatened morale. But health officials inspected every building in Chinatown, cleaned the streets, hauled away garbage, washed down sewers and cellars. The officers of the Marine Hospital Service worked with them, and the Surgeon General offered antiplague serum and vaccine. He was "not apprehensive about immediate danger," he said, but hoped to "prevent establishment of a plague focus."

Boiling out of China in 1894, plague had become epidemic on every continent except North America within 5 years. India had reported 135,000 deaths for 1899. Wherever plague appeared, commerce and travel suffered. No one understood how to control it. People knew that rats died from it, but not how fleas carried the germs from rats to humans. Health authorities believed that personal items, such as clothing, conveyed infection, hence they emphasized finding cases and cleaning the environment.

But Chinatown defied cleaning. Claptrap

buildings lacked light, ventilation, and sanitation. Gross filth accumulated in miserable streets and alleys. A contemporary report said, "Sewers are used as subways from one house to another. Cellar dwellers inhabit their caverns, and so far as anyone knows, they are burrowing, excavating, and maintaining their usual subterranean communications, rendering it not only easy to pass from one portion of a block to another, but forming at the same time an easier way for rats to gain access to the houses . . . . The ramifications of a house which has been orientalized are so great and so tortuous as to be utterly beyond the conception of the occidental mind." The *British Medical Journal* said, "The Chinese community in San Francisco . . . seems to form a kind of *imperium in imperio*, if Mr. Bryan will excuse the expression. How many Chinamen have died of the plague, how many corpses of such persons are now awaiting shipment to China . . . no white man knows, or ever will know."

This was what worried the health authorities. By the third week in March, still "pegging away at the plague," as a newspaper scoffed, they had found only two suspected cases. Conviction grew that the Chinese were smuggling sick out of the city. Officials examined every Chinese they could find attempting to leave. Surgeon A. H. Glennan, inspecting the Marine Hospital Services' activities, reported that the death rate in Chinatown was only half what it should be. The Chinese, he believed, were secretly disposing of corpses.

At the end of March, health officials concluded the second campaign. Newspapers jibed, "Opera bouffe of the bubonic plague has come to an end, the curtain has been rung down." It remained down for more than a month.

Then, on May 15th, Dr. Kinyoun wired the Surgeon General that there had now been four deaths from plague, with no connection between cases. He regarded the disease as epidemic. The Surgeon General responded by ordering four officers to San Francisco and authorizing Kinyoun to employ other physicians temporarily. He got the Secretary of the Treasury (the Marine Hospital Service was in that Department) to ask President McKinley for authority to apply the strict quarantine laws of 1890, and the President assented on May 21st. From then on, no Asiatics could leave California unless certified by Kinyoun's officers as unlikely to convey infection to other communities. Inspectors covered all border points.

City health authorities began a third house-to-house search, again within a police cordon. They offered plague vaccine, which the Chinese refused. As the inspections proceeded, officials noted increasing resistance, and on May 24th a Chinese organization called the Six Companies petitioned the U. S. Circuit Court to restrain the authorities from interfering with the freedom of passage of Chinese in California. Four days later, Judge William W. Morrow declared the quarantine illegal, ruling that only the board of supervisors, not the board of health, could issue such regulations. The supervisors acted that evening, ordering an even tighter cordon around Chinatown.

A week after Judge Morrow's decision, the health authorities, dismayed by the crowding and filth in Chinatown, determined to move 7,000 Chinese into camps on Angel Island or Mission Rock. But Judge Morrow promptly issued an order forbidding detention, and on June 14th he directed abandonment of the Chinatown sanitary cordon.

Meanwhile, Kinyoun reported attempts to bribe him and threats to run down his official launches in the bay. On June 16th, the law reached out for him. A Chinese, Wong Wai, filed an affidavit charging that Kinyoun had prevented him and others from leaving San Francisco, in defiance of the injunction of May 28th. Judge Morrow ordered Kinyoun to answer to a contempt citation.

Kinyoun regarded his predicament with gloom. He had no confidence in the district attorney, who was to defend him. He asked for additional counsel; when that request failed, he urged Dr. Wyman to arrange for the Justice Department to appeal his anticipated conviction. He had little reason to expect sympathy from Judge Morrow, who had ruled against the health authorities in three suits. In granting the injunction of June seventh, the judge had said, "If it were within the province of this court to determine this issue, I think . . . . I should be compelled to hold that plague did not exist and has not existed in San Francisco." Judge Morrow had leaned toward the testimony of physicians who practiced in Chinatown. Kinyoun believed that some of them "are nothing more nor less than vampires, who engage in the work simply for the living that is in it, and will do anything to curry favor with their patients."

While Kinyoun uneasily awaited his trial, the Surgeon General decided to stop inspections of

Chinese attempting to leave California, "developments not having demonstrated the necessity for continuance of restrictions on travel." His orders went into effect on Sunday, June 24th.

In San Francisco, Service officers were conducting inspections in the Market Street Ferry Building, which seemed to Assistant Surgeon Kerr "the busiest place in California." Within a few hours after receipt of Dr. Wyman's order, he and Assistant Surgeon Lumsden "were stretched out on the lawn of the Monterey Hotel fifty miles away." It was the first time, Kerr said, that he "had been out of sight of the San Francisco waterfront for more than two hours at a time during daylight." He was soon to leave for duty at Hongkong. Enroute he would see at Honolulu the smoking ruins of the Chinese quarter, destroyed by the authorities trying to halt plague. In Hongkong his neighbors at the hotel would die of plague. Later, after moving into safer quarters, he would find plague corpses tossed into the compound during the night.

Kinyoun's trial opened on June 25th. While it was in progress, the Surgeon General reported that the Hygienic Laboratory in Washington had confirmed Kinyoun's diagnosis of the animals inoculated after Chick Gin's death. As June and Kinyoun's trial drew to a close and the campaign came to a standstill except for desultory measures to reduce the rat population, the number of plague cases stood at ten.

Judge Morrow, ruling in Kinyoun's trial on July third, reviewed Wong Wai's claim that steamship agents had acted on Kinyoun's orders when they refused him passage on the steamship *Orizaba* to Eureka, California. Kinyoun had no jurisdiction within the state, the judge said, and restricting "a particular class of persons, as Chinese or Japanese, and not . . . others similarly situated" was discrimination against individual liberty. Judge Morrow noted Kinyoun's denial that he had attempted to limit travel within the state and, accepting this testimony, decreed that Kinyoun's orders regarding persons leaving San Francisco for other states and territories did not violate his injunction.

Kinyoun wired the Surgeon General "Court decides me not in contempt . . . ." Then he settled down to "watching the situation . . . to inaugurate or assist in measures which may be necessary or practicable." This, under the circumstances, meant essentially no measures at all.

By October, reported plague cases had reached 19 and officials were still watching. One who became impatient was the governor of Texas, Joseph D. Sayers, who called on the Surgeon General early in the month. His skepticism appears in a later communication to Wyman: "I have but little confidence in the California authorities, and unless I have positive assurance from you that this State [Texas] will be fully protected by you from the disease, I shall immediately order the most stringent quarantine restrictions." He did, too, and so did Colorado, Mexico, Ecuador, and Sydney, Australia.

This was not the first time that Texas had excluded travelers from California. During the previous June, the Surgeon General had wired Health Officer Blount at Austin: "Will you not kindly admit Assistant Surgeon Robert Wilson . . . coming from San Francisco . . . now detained Deming, New Mexico . . . by your inspectors?" Young Dr. Wilson's object was matrimony, and apparently Texas was reluctant to hinder romance, for his mission was accomplished.

Soon after the visit from Governor Sayers, the Surgeon General asked Kinyoun for a report. Kinyoun replied that Health Officer O'Brien "admitted to me that so far the health department had been absolutely powerless to enforce the sanitary regulations in Chinatown or make any progress toward eradicating the infection . . . . I am at the point sometimes of stating that plague will exist . . . until the district now occupied by the Chinese has been depopulated and destroyed."

After Christmas, Dr. Wyman sent Surgeon Joseph H. White to San Francisco to "examine into any matters . . . which may require your attention." Dr. White's first report, on January eighth, 1901, probably did not surprise his chief: "Think representatives of Six Companies, commercial interests of San Francisco, and press are all in unison; perhaps friendly to Service, but inimical to Joseph J. Kinyoun, but doubt even former." Next day, after investigating with Kinyoun and Kellogg a suspected plague case, he wrote, "I desire to add my testimony . . . to that of the others . . . it is my honest opinion that this man died of true bubonic plague . . . . I make this statement because various inuendoes have been put forth to the effect that possibly these corpses were inoculated with bacilli . . . ."

At this point, a new character, destined to assume a prominent role in the action, demands attention. He is the Governor of California,

Henry T. Gage. In June, 1900, he had denied the existence of plague in California. Now, in January, 1901, he attacked Kinyoun in his address to the legislature, declaring him incompetent as a quarantine officer and ignorant in respect of plague. In a roundabout manner, the Governor suggested that the animals Kinyoun had reported as developing plague after inoculation, really had been infected with cultures from the laboratory. He did, however, use the words "innocently" and "accidentally." A San Francisco newspaper reported the address under the heading "Governor Gage Pleads for Economy and Denounces the Plague Fakers."

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Both Kinyoun and White wanted action, and on January 19th the Secretary of the Treasury, Lyman T. Gage, on the Surgeon General's recommendation, appointed a Scientific Commission to investigate reports of plague in San Francisco. Senator George C. Perkins of California wired Governor Gage (he and the Secretary had the same last name) that members of the Commission were "of highest possible standing . . . [and were] instructed to be unprejudiced and independent." They were Simon Flexner of the University of Pennsylvania, F. G. Novy of the University of Michigan, and Lewellys F. Barker of the University of Chicago. All were physicians familiar with plague.

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but humble request be denied us, then we respectfully submit that before the Commissioners' report be made public, and prior to taking any action thereon, . . . representatives of this state . . . be accorded a formal hearing in Washington . . . no one can honestly be of the opinion that [plague] is epidemic in San Francisco nor can anybody seriously contend that ample protective . . . measures can not be taken . . . without spreading alarm . . . or . . . disturbing our commercial affairs."

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and punishing the alleged slave traffic in Chinese girls . . . may properly be resumed."

Chinatown dismayed White as it had his predecessors. "There has probably been an average of two carloads of filth removed from each house, and much more remains," he wrote early in the campaign. "Some of these rooms, practically underground and separated from either street or alley by flanking rooms on either side, . . . are dirty beyond anything that may be imagined . . . filled with a stifling odor suggesting months of opium smoking, with occasional urination on the floors . . . it is the accentuated odor of a rat den."

By the end of May, the Surgeon General felt that the California authorities had probably complied with the agreement made by the Governor's Commission. Flexner had told him that perhaps plague was disappearing. He recalled White to Washington. On June tenth, Passed Assistant Surgeon Rupert Blue took charge in San Francisco (11 years later he became Surgeon General after Wyman died), and on June 21st he reported completion of the disinfection of Chinatown.

The Surgeon General later said that "it appeared possible for a time that plague had been successfully eradicated from San Francisco." He and Governor Gage agreed that the Service would maintain a laboratory under Dr. Blue who, with two assistants, would perform autopsies on all suspected deaths and inspect Chinatown twice daily "for a short time to come."

At the end of June, Dr. Kinyoun received orders to survey the Service's operations in the Far East. This was his last assignment, for upon his return he resigned. Before his detail to San Francisco, he had been Director of the Hygienic Laboratory, since its beginning in 1887. This institution ultimately became the National Institutes of Health. Kinyoun bears the distinction of being the first among the men who have directed this famous research center.

Between July and October, 1901, 16 more plague cases brought the total to 50. At the end of the year, Dr. Mark J. White, who succeeded Blue (who had succeeded Joseph White), reported that "the opposition by the State Authorities, the commercial interests, and the Chinese to our plague work here still continues, more solidly, perhaps, on the part of the latter than formerly, and surely no less on the part of the others." Matters stood this way for another ten months, until October, 1902, when the record of plague cases reached 89.

Then, at last, events began moving toward a climax.

The Surgeon General, expressing "apprehension on the part of the Service," sent Dr. Glennan to California for the second time. Glennan called on Governor Gage on October 15th.

"He received me pleasantly," Glennan reported. "He stated his belief that the disease in Chinatown was a syphilitic septicemia . . . he was convinced of our sincerity, and stated that he would render every aid possible." Even the patient Wyman must have tired of such talk by now.

Decisive action began not in California, but in Connecticut. There, at the end of October, the State Boards of Health held their annual conference. They spent much of the time registering concern over the situation in California. Word of their dissatisfaction reached San Francisco promptly. "The executive officials . . . were greatly agitated by the fear of quarantine against California by the other states," the Surgeon General said. He considered this "unnecessary alarm" but confirmed that there had been a "suggestion relating to . . . calling . . . another conference" to deal exclusively with plague.

California officials conferred on November third, assuring Dr. White that the State Board of Health was "willing to institute throughout California any reasonable recommendations of the Surgeon General." Glennan wired the following day, "Situation here approaching cooperative action . . . . Hope other states will not take precipitate action against California."

Within a few days, the Governor appointed Dr. Matthew Gardner, chief surgeon of the Southern Pacific Railroad, to accompany Glennan in searching for plague in cities other than San Francisco. Both as Governor Gage's representative and later, Dr. Gardner worked amicably and effectively with officers of the Service, until his sudden death from appendicitis.

San Francisco health authorities began serious collection and destruction of rats in November. By Christmas, laboratory workers had found plague in 16 of nearly 500 rats examined.

During the week before Christmas, 1902, Dr. Wyman visited San Francisco, and made a night inspection of Chinatown. Afterward, he said that his "personal observation confirmed the

fact that the efforts of the City Health Officials . . . in the eradication of plague . . . had been carried on under unusual difficulty and lack of support from the then existing State Health Authorities." These Authorities were not to exist much longer. Perhaps the most significant act of the Surgeon General's visit was an informal consultation with the man who soon would exert a major influence on events, George C. Pardee, M.D., the Governor-elect of California.

The outgoing governor presented his last message to the legislature on January sixth, 1903. Glennan wired the Surgeon General, "Governor Gage's final message . . . reiterates his position. Attacks Kinyoun and press publishing false epidemic predictions. Wordy denial plague situation."

Two weeks later, the State and Territorial Health Officers, at their request, met with the Surgeon General. They declared that "the presence of plague in California is established beyond doubt . . . the gravity . . . is greatly increased by the gross neglect of official duty by the State Board of Health . . . and the obstructive influence of the recent Governor . . . [and] by the failure of the City Government of San Francisco to support its City Board of Health. The conference will consider the safety of the country assured as soon as satisfied that a competent Board of Health of San Francisco and a competent State Board of Health, in cooperation with the United States Public Health Service, [the official name of the Service at that time was United States Public Health and Marine Hospital Service] will proceed under definite, harmonious, and effective laws and ordinances . . . provided with ample funds, and . . . in free exercise of their lawful powers."

Late in January, newspapers quoted the new Governor as declaring, "Whatever the Marine Hospital service desires me to do in the way of public health preservation shall be done . . . . The medical authorities have emphatically declared that plague has existed and does exist in San Francisco, and that settles it as far as I am concerned . . . ."

On February second, in San Francisco, a "mercantile joint committee" strongly urged official action "to the end that all danger from the bubonic plague may be eradicated . . . that the confidence of the Boards of Health of the other States and Territories may be restored . . . that no injury may result to . . . commerce." The Governor, Mayor Schmitz, and members of the Committee signed a statement assuring "complete and harmonious action by all the health authorities." Doctors Glennan, Gardner, and O'Brien prepared plans for "supervision and sanitation of the Chinese district." The campaign, to last at least a year, would be under direction of the Public Health and Marine Hospital Service. Cities throughout California were to watch for Chinese from San Francisco who might attempt to hide. As the new campaign began, the total of plague cases had reached 93.

In May, Dr. Blue returned to take charge, relieving Glennan. In his report at the end of June, Blue expressed satisfaction at being able to enforce "more radical measures than had hitherto been practicable." The most important of these, he felt, was "condemning and destroying insanitary wooden additions erected by Chinese tenants on the sides and roofs of the original buildings."

The last case of plague, reported on March first, 1904, brought the total to 120. Almost exactly 4 years had passed since the death of Chick Gin. The campaign in Chinatown, which lasted longer than the War Between the States, ended officially with the closing of the Federal laboratory on April 13th, 1905. The final report concludes, "The united and harmonious efforts of the Public Health and Marine Hospital Service, the Governor and health officials of California, and the municipal and health officials of San Francisco, have brought to a determined and successful completion the work of eradicating plague in the Chinese district of San Francisco."

Nothing remains of that execrable Chinatown. The fire of 1906 consumed it all, including Dupont Street, where Chick Gin and others of his countrymen died of plague. In today's Chinatown, this once iniquitous thoroughfare bears the name Grant Avenue.

Plague reappeared in San Francisco after the fire, but a vigorous campaign brought it under control. In time, foci of infection appeared elsewhere, and still persist, but that is another story.

The turmoil which followed Chick Gin's death was totally unnecessary. What caused it was not plague, but ignorance, prejudice, and stubbornness; especially stubbornness.

ACKNOWLEDGMENTS

I am indebted to Dr. John W. Kerr and Dr. Charles Armstrong for advice and assistance in the preparation of this article.

OBSERVATIONS ON TREATMENT OF INFANT DIARRHEA IN PANAMA

MARK T. HOEKENGA

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Infant diarrhea is one of the most common and most serious medical problems in warm climates all over the world, and the hot, humid, banana-producing lowlands of western Panama are no exception. Here, diarrhea vies with respiratory-tract infections as the major cause of morbidity in infants. As a cause of death in this age group, it is undisputably first.

There are various causes of infant diarrhea, the principal etiologic factors varying from place to place and from time to time. In western Panama, the relative importance of enteric viruses, including the *ECHO* group, is not known. Nor has work been done to elucidate possible enteropathogenic *Escherichia coli* or other pathogenic variants of normal intestinal flora. We do know, though, that shigellosis and salmonellosis are not usually significant, that *Entamoeba histolytica* is uncommonly involved, and that the incidence of intestinal worms (40%) is approximately the same in diarrheic and nondiarrheic stools. In a preliminary study, cultures of stools from 50 consecutive infants with diarrhea revealed heavy growth of organisms of the coli-aerogenes type in 96%, *Shigella* in only 4%; results of cultures of normal-appearing stools from other infants without diarrhea were approximately the same. The most commonly encountered organisms in both series, in order of frequency, were *Escherichia communior*, *Aerobacter aerogenes*, *Pseudomonas aeruginosa* and *Esch. coli*. We suspect enteropathogenic types of some of these organisms to be involved in our cases. Much has been written on this subject for other areas and has been excellently summarized by Neter.[1] Poor nutrition is not uncommon in the area of this study, and may be a factor in some cases.

Like many clinicians in other locations, we have not been completely satisfied with the commonly accepted remedies such as succinylsulfathiazole, bismuth, paregoric or kaolin mixtures, nor with the withholding of food by mouth. We wished to test therapeutic agents which were potentially more effective. Also, we shared the idea with others that the prolonged withholding of food in the treatment of diarrhea serves no good purpose unless there is associated vomiting, and we wished to test this precept in a field study. The presently reported observations were undertaken for these reasons.

MEDICATIONS

Two of the antibiotics which are effective against intestinal pathogenic bacteria are neomycin and streptomycin. Neomycin is active against a broad range of gram-negative organisms, is effective against hemolytic *Staphylococcus aureus* and other staphylococci, but inferior against hemolytic streptococci. Streptomycin is also effective against a wide variety of pathogenic gram-negative and gram-positive bacteria, the effectiveness varying with different strains. Neomycin and streptomycin together have a broad antibacterial spectrum against intestinal pathogens, without toxicity from usual oral doses, and rarely with development of sensitivity. We decided to make a comparative study of certain preparations containing these antibiotics, and one of the frequently used succinylsulfathiazole compounds. The medications used were neomycin sulfate*, succinylsulfathiazole†, and a combination of streptomycin sulfate (15 mg) and neomycin sulfate (5.25 mg) in 1 gm of Carobtree base.‡

The dosages used were as follows:

Neomycin preparation: 25 mg neomycin per pound of body weight per day, divided into 4 to 6 daily doses (Prep'n. A).

Succinylsulfathiazole preparation: 15 to 135 ml daily (depending upon size and age of child), in 6 to 8 divided doses (Prep'n. B).

* Mycifradin,® Upjohn. This contains 125 mg neomycin sulfate per 5 ml of a flavored vehicle.

† Cremosuxidine,® Merck, Sharpe & Dohme. This is a suspension containing 10% succinylsulfathiazole with pectin and kaolin.

‡ Intromycin,® Pitman-Moore Co. The base of Intromycin is a powder made from the pulp of the beanshaped fruit-pod of the carob tree, a leguminous plant native to Mediterranean shores, but now common in many other locations. This dried, powdered pulp has been shown by a number of investigators in Europe and the United States to possess merit in the control of diarrhea, presumably because its high content of pectin, lignin and hemicellulose gave it great absorptive and water-binding properties as well as a buffering action in the intestines.

Preparation containing streptomycin and neomycin: 20 to 120 g of the powder daily (depending on size and age of child), in 6 to 8 divided doses, suspended in milk or in water (Prep'n. C).

Dispensators were instructed to advise parents of the sick children that strict dieting was not necessary unless vomiting was a problem. Almost always, milk and other infant foods were continued in the accustomed way.

MATERIAL AND METHODS

Treated cases. The three medications were distributed to six field dispensaries located in the banana labor camps of the Chiriqui Land Company (United Fruit Co.) in western Panama. Dispensators were not informed of the constituents of the compounds, nor given any clue as to their expected performance. They were also forbidden to discuss with each other any aspect of the experiment and its results until the study had been completed. Care was taken to include only children with actual diarrhea, not those with mere "loose bowels" or "green stools," frequently referred to by the parents as diarrhea. The severity of the diarrhea was rated as mild, moderate or severe, depending upon the number of stools, the duration of the disease, presence of fever, presence of dehydration and the general appearance of the child. Approximately two thirds of all infants treated had "moderate" diarrhea, one sixth "mild" diarrhea and one sixth "severe." The most severe cases, however, were not treated in the dispensaries, but were hospitalized. They will be mentioned separately when considering the results.

All of these more-severely-affected patients had some degree of dehydration and acidosis, usually high fever, and vomiting. Fluid and electrolyte replacement were instituted immediately and within a few hours Prep'n. C was started, first suspended in water, but after one to three feedings directly in the milk.

Tests in adults. Since diarrhea in adults is not apt to be such a fulminating and potentially fatal entity, we did a simple, related but not strictly comparable, study in them rather than in infants. One hundred and twelve diarrhea patients ranging in age from 13 to 68 years were treated with one of three identical-appearing tablet preparations. One of the tablets was a lactose placebo; one contained iodochlorhydroxyquin, 375 mg; and the third contained neomycin sulfate, 375 mg, kaolin 120 mg and pectin 5 mg. The three compounds were code-labelled, and we ourselves did not know the identity of them until the study had been completed and reported to the supplier of the tablets (The Upjohn Co.). The dosage in all cases was two tablets four times daily. The same criteria for cure were used as described above.

RESULTS

Treated cases. The results of treating 261 cases of infant diarrhea with these three compounds are presented in Table 1. For the purpose of this study, a classification suggested by Kaliski and Mitchell[2] was employed. Response was considered "good" if the stools became thicker and frequency diminished markedly within 48 hours. The response was classified as "fair" if these changes occurred within 48 to 96 hours, and "unsatisfactory" if the improvement was not obvious within 96 hours. Using these criteria, Table 1 presents the surprisingly good results obtained with all three medications, and especially with Prep'n. C. With the latter compound, 88.1% of 126 infants were cured within 48 hours and another 9.5% within a second 48-

TABLE 1

Dispensary treatment in 261 cases of infant diarrhea

Result	Prep'n. A		Prep'n. B		Prep'n. C	
	No.	%	No.	%	No.	%
Cure in less than 48 hours	71	74.0	31	79.5	111	88.1
Cure within 48–96 hours	21	21.9	6	15.4	12	9.5
More than 96 hours required for cure (unsatisfactory result)	4	4.1	2	5.1	3	2.4
	96		39		126	

TABLE 2

Hospitalized cases of infant diarrhea treated with Prep'n. C

Result	No.	%
Cure in less than 48 hours	10	50
Cure within 48–96 hours	8	40
More than 96 hours required for cure (unsatisfactory result)	2	10
	20	

hour period. Unsatisfactory results were uncommon with all three compounds. In one of the failures of Prep'n. C and one of the failures of Prep'n. A, *Giardia lamblia* was present and considered a probable reason for the diarrhea and for the failure to respond to the treatment employed; cure was obtained with quinacrine. The cause of unsatisfactory response in the other instances was not apparent, but all of these infants were cured with more prolonged treatment.

Ten of the 20 hospitalized cases were cured within 48 hours and eight within 48 to 96 hours (Table 2). One baby died 2 hours after admission and another was cured only after several days of intensive therapy; these are listed as the two "unsatisfactory" cases.

No toxic effects were noted during this study of these compounds, during either dispensary or hospital use; nor was any complication encountered as a result of continued feeding in the nonvomiting cases.

Tests in adults. Of 37 patients who took the placebo 92% were cured within 48 hours; of 40 who took the iodochlorhydroxyquin 85% were cured within 48 hours; and of 35 individuals in the neomycin group 83% were cured within 48 hours.

COMMENTS

Treated cases. The majority of our infant patients responded well to either of the three compounds tested and it is naturally wondered how they would have done without drug treatment. It can safely be surmised that most of the cases would have been self-limited and would have improved with time. But because diarrhea is the primary cause of infant death in this area, we can also be reasonably sure that in any untreated control groups additional deaths would have occured. Hence, a strictly control study was deemed inadvisable.

The results obtained with Prep'n. C were significantly better than those obtained with the other two medications. Suggested reasons for this are, first, that carob flour is an effective antidiarrheic, as indicated by an extensive literature. Because it is rich in pectin, lignin and hemicellulose, it has great absorptive and water-binding properties. It buffers intestinal contents, absorbs irritant secretions, bacteria and toxins, tends to prevent dehydration and the loss of electrolytes. In addition, additive and synergistic effects of neomycin and streptomycin have been demonstrated against some strains of *E. coli* and other organisms. The addition of small amounts of neomycin to cultures containing insufficient streptomycin to suppress bacterial growth resulted in complete inhibition of growth.[3] Furthermore, cross-resistance between streptomycin and neomycin occurs to only a limited extent, so that most strains of organisms resistant to one of these antibiotics will be found sensitive to the other.[3]

It is not possible to assess the exact effect of the policy of not withholding food. However there was no evidence that this was harmful, and there were numerous instances in which the continued feeding of infants appeared distinctly and rapidly beneficial.

Tests in adults. The obvious conclusion to be drawn from the tests in adults is that the vast majority of diarrhea in adults in this area is self-limited and really needs no treatment. It is dangerous to project these results into our thinking about infant diarrhea, and we have no intention of doing so, but the data are intriguing nevertheless. Even though stool cultures reveal the same organisms to be present in infant and adult diarrhea these conditions behave as distinct entities clinically.

SUMMARY

A study of 281 cases of infant diarrhea is reported. Of these, 261 were treated with drug preparations containing either neomycin, succinylsulfathiazole or a combination of neomycin and streptomycin on an outpatient, dispensary basis. The other 20 were hospitalized. Results with all three compounds were good, but especially so with the neomycin-streptomycin combination. With the latter compound, 88.1% of infants were cured within 48 hours and another 9.5% within a second 48-hour period.

A related study of diarrhea treatment in adults revealed that a placebo gave results equal to treatment with iodochlorhydroxyquin or with tablets containing neomycin sulfate 375 mg, kaolin 120 mg and pectin 5 mg.

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THE SEROLOGICAL DIAGNOSIS OF EOSINOPHILIC LUNG (TROPICAL EOSINOPHILIA) AND ITS ETIOLOGICAL IMPLICATIONS*

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Since the recognition by Frimodt-Möller and Barton[21] and Weingarten[51] of a syndrome characterized by paroxysms of cough with hypereosinophilia responding favorably to treatment with organic arsenicals, many reports have appeared from India, Ceylon, and other tropical regions confirming the existence of this clinical entity, variously designated as eosinophilic lung, tropical eosinophilia or pulmonary eosinophilosis. The clinical picture is distinct in the majority of these patients, but similar symptoms with hypereosinophilia may sometimes be produced in the course of known parasitic infections such as strongyloidiasis,[14] bilharziasis,[33] ascariasis,[3] visceral larva migrans,[4] and pulmonary gnathostomiasis,[41] and in allergic conditions such as Loeffler's syndrome and some cases of bronchial asthma.[8] This has made the diagnosis of eosinophilic lung difficult except on the basis of the relief of symptoms and hematological response following arsenotherapy or treatment with diethylcarbamazine. Although hypereosinophilia is a cardinal feature of this condition, the degree of eosinophilia considered to be of diagnostic significance has varied from worker to worker. Minimal acceptable counts have been placed variously at 5,000, 3,000 and 2,000 eosinophils per cu. mm. Other workers have accepted an eosinophilia of as low as 1,000 cells per cu. mm as diagnostic, but have had therapeutic failures in these patients. Previous experience in Singapore has shown that an eosinophilic leukocytosis of 3,000 or more cells per cu. mm is a constant finding in cases of eosinophilic lung,[10, 13] and this figure is regarded by the present authors as being of diagnostic significance. A laboratory test that would assist in the differentiation of eosinophilic lung from other groups of eosinophilias would, therefore, be useful in diagnosis.

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Reports on eosinophilic lung up to date have emphasized the lack of a demonstrable etiological agent. Recently, the successful results obtained by the use of diethylcarbamazine in the treatment of this condition,[22, 11, 7] suggested the possibility that the causative agent may be some form of filarial worm. Clinically there was no evidence to support this; in 110 patients cured with this drug[13] there were neither history of filariasis nor signs of infection and no microfilariae were found in blood samples taken at night, using Knott's concentration technique.[31] It was, therefore, decided to examine the sera of subsequent patients for filarial complement-fixing (CF) antibodies, using as antigen a 1% alcoholic extract of dried powder of *Dirofilaria immitis*. The results obtained from tests of sera of 12 patients indicating that the filarial complement fixation test (FCFT) may be useful in the diagnosis of eosinophilic lung were reported in a preliminary communication.[12] The present paper reviews the results of this test carried out on a larger series of patients.

MATERIAL AND METHODS

The FCFT was carried out on the following study group:

1. 228 persons with hypereosinophilia of 3,000 or more cells per cu. mm.
2. 85 persons with mild eosinophilia of varying degree, the counts ranging from 500 to under 3,000 cells per cu. mm.
3. 119 cases of human filariasis.
4. 33 dogs with *D. immitis* infection.
5. 30 children suffering from severe ascaris infection.
6. 160 normal individuals who had no eosinophilia and no clinical or parasitological evidence of filariasis.

In none of the patients in Groups 1 and 2 was there evidence of past or present filarial infection, and parasitological examinations of sputum and blood samples taken at night (once in each case using Knott's concentration technique) were negative for microfilariae.

The 30 children in Group 5 each passed some

30 to 50 round worms after treatment. Although 14 of these children had an eosinophilia, in none of them did the count reach 3,000 cells per cu. mm.

Group 1 cases were given treatment with diethylcarbamazine, the dosage being 6 mg per kg body weight three times a day for 5 days. After commencement of treatment, further blood samples for the FCFT were taken and leukocyte counts done at weekly and then at fortnightly intervals.

Technique of FCFT. The worm material used in antigen preparation was supplied by Professor Paul C. Beaver, Tulane University, New Orleans, La. Living *D. immitis* adults were washed in saline, cut up finely with scissors, and homogenized in distilled water in a Waring-Blendor. This material was filtered through gauze, dispensed into ampoules, shell-frozen in alcohol-CO_2-ice, then dried and sealed *in vacuo*. Each ampoule contained approximately 0.1 g of dried material equal to six worms.

A 1% alcoholic extract of the dried worm material was prepared in 96% ethyl alcohol according to the method of Fairley[16] except that the extract was not further concentrated.

A preliminary survey of sera from patients with bancroftian filariasis and dogs with *D. immitis* infection was carried out to find a positive serum for titration reference. This showed that a 1:10 dilution of the extract as prepared above had very weak antigenic properties. For this reason, 0.4 ml of a 1% solution of cholesterol in absolute alcohol was added to every 0.6 ml of antigen extract. Precedence for this method of antigen sensitization has been well established in the preparation of Wassermann antigen.[30] The antigenic properties of the cholesterolized extract were found to be distinctly enhanced. Its antigen titer was computed to be 1:120, and it was shown to be neither anticomplementary nor hemolytic in a 1:10 dilution. Wassermann-positive sera gave no reaction with this antigen.

Blood specimens were centrifuged and sera removed within 24 hours of collection. After inactivation for 30 minutes at 56°C, sera were kept in a deep-freeze chest until testing. They were reheated to 56°C for 10 minutes before the test, which was performed within 5 days after blood was drawn.

In the main test, each specimen of serum was diluted 1:5, 1:10, 1:20, 1:40, 1:80, 1:160, and 1:320. Antigen prepared according to titer, 3 minimal hemolytic doses (MHD) of complement, and 3 MHD of amboceptor were used with a 3% solution of thrice-washed sheep's erythrocytes. Of each of the various serum dilutions 0.11 ml was mixed with 0.11 ml of antigen and 0.11 ml of complement. After incubation for 1 hour at 37°C in a water-bath, 0.22 ml of the hemolytic system was added. The tubes were reincubated for 45 minutes at 37°C after which a preliminary reading was made. Serum controls and negative sera showed complete hemolysis. The decisive reading was taken the next morning after overnight storage at room temperature. Only those reactions in which absolutely no hemolysis occurred were reported as positive.

RESULTS

The initial FCFT carried out on the 228 persons with hypereosinophilia in Group 1 gave positive titers in 206 cases (90.4%). On the criterion of adequate symptomatic and hematological response following treatment with diethylcarbamazine, 213 (93.4%) of the 228 persons were cases of eosinophilic lung (EL). The remaining 15 are referred to collectively as non-EL cases. The results of the serological findings are summarized in Table 1. The FCFT positive rate is 95.3% among EL cases as compared with 20% among the non-EL cases in this group.

Of the ten EL patients who were initially FCFT negative, four showed positive titers when the test was repeated 2 weeks after commencement of treatment. These positive readings were 1:10, 1:20, 1:20 and 1:40, respectively; the titers later declined and became negative. The majority of the other 203 EL patients had high initial titers and in only eight instances were low titers of 1:5 obtained.

The initial FCFT titers of the EL and non-EL cases in Group 1 are compared in Table 2 with the titers obtained from similar tests carried out

TABLE 1

FCFT results in EL and non-EL cases (Group 1)

	EL	Non-EL	Total
FCFT			
Positive	203	3	206
Negative	10	12	22
Totals	213	15	228

TABLE 2

Initial FCFT results for the different study groups

FCFT titers	Study groups						
	1 Hypereosinophilia		2 Mild eosinophilia	3 Human filariasis	4 Dog filariasis	5 Ascariasis	6 Normal
	EL	non-EL					
Negative	10	12	79	95	26	28	151
1:5	8	1	2	10	4	1	4
1:10	23	2	2	7	—	1	4
1:20	44	—	2	7	3	—	1
1:40	51	—	—	—	—	—	—
1:80	43	—	—	—	—	—	—
1:160	20	—	—	—	—	—	—
1:320	14	—	—	—	—	—	—
Totals	213	15	85	119	33	30	160
% positive	95.3	20.0	7.1	20.2	21.2	6.7	5.6

TABLE 3

Effect of treatment on FCFT titer and esoinophil count in cases of eosinophilic Lung

Weeks after commencement of treatment	% of cases becoming negative to FCFT (87 cases*)	% of cases in whom eosinophil count had fallen to <3,000 cells per cu. mm (95 cases)
1	2.3	17.9
2	9.2	53.7
3	11.5	77.9
4	26.4	90.5
6	50.6	95.8
10	75.9	100.0
15	86.2	100.0
20	90.8	100.0
25	94.3	100.0
30	95.4	100.0

* Excludes 8 cases whose initial titers were negative.

on the five control groups. Several striking features are noticeable in these figures. Firstly, the FCFT is positive in 95% of the EL cases, a positive rate which is greatly in excess of those for the other groups. Secondly, among the other groups there appear to be two distinct levels in their positive rates: 20 to 21% in study Groups 3 and 4 and non-EL cases of Group 1, and 6 to 7% in Groups 2, 5 and 6. Thirdly, most of the titers obtained in the EL cases are much higher than those found in sera from cases of human filariasis and even exceed those found in sera from infected dogs where the antigen used is homologous.

After treatment with diethylcarbamazine, FCFT titers gradually diminished in all the EL cases who were initially positive. In some instances there was an initial rise in titer immediately following treatment, before decline occurred. The duration of time (in weeks) taken for FCFT titers to become negative and for eosinophil counts to fall below 3,000 per cu. mm are summarized and compared in Table 3. These figures are based on 95 cases who had completed the full observation period of 30 weeks and who were given one course of treatment with diethylcarbamazine. Eight of these cases had negative titers at the commencement of treatment, and are therefore excluded from the figures in the column referring to FCFT response. It can be seen that the fall in eosinophil counts to below 3,000 per cu. mm occurs much more rapidly, on the average, than the fall in the FCFT titers. With few exceptions, the titers became negative within the total observation period, about half of them becoming negative within 6 weeks (the median duration). Subsequent tests showed that the titers which had become negative following treatment have since remained negative.

The 15 non-EL cases with cough and hypereosinophilia in Group 1 showed no symptomatic or hematological response to diethylcarbamazine therapy. A diagnosis of visceral larva migrans was made in 12 of them who were children, but, until larvae can be demonstrated in tissue obtained by biopsy or at post-mortem, this can be only presumptive. However, it is of significance that proven cases of visceral larva migrans do not respond to treatment with organic arsenicals or diethylcarbamazine[48] although during and for a period following, the administration of the latter there may be marked fluctuations in the levels of eosinophilia.[54]

The mild eosinophilia observed in the 85 persons in Group 2 was probably due to ascaris or hookworm infection or to allergy. The majority of these patients had no symptoms and 11 who were treated with diethylcarbamazine showed no hematological response.

DISCUSSION

Diagnosis. The large percentage of positive reactions (95%) obtained in the group of eosinophilic lung cases is in strong contrast to the

negative results obtained in most of the non-EL, mild eosinophilia, ascariasis and normal control groups. The very small number of positive reactions (6.9% or 20 out of a total of 290 sera) obtained in these control groups, together with the relatively low titers, can be explained as possibly being due to latent filariasis during the prepatent stage, when microfilariae are absent.[42] That the positive reactions became negative when ten of these patients were treated with diethylcarbamazine, an antifilarial drug, is significant and supports this premise.

The predominantly negative reactions (93.3%) in the sera obtained from 30 children heavily infected with *Ascaris lumbricoides* show that this infection does not influence the results of the FCFT. According to Minning and McFadzean[27] there is no evidence that ascaris infection affects the FCFT.

In the past, the differentiation of one group of of eosinophilias, called eosinophilic lung, from other eosinophilias was mainly a therapeutic one as shown by response to treatment with organic arsenicals or diethylcarbamazine. On the basis of the above findings, the FCFT can now be used as an aid in differentiating eosinophilic lung from the remaining eosinophilias.

Although cough with or without breathlessness has been generally accepted as the main presenting symptom in cases of eosinophilic lung, reports have appeared from time to time suggesting that the clinical picture is more variable; the only constant feature being a high eosinophilic leukocytosis. Atypical presentations without cough, but with symptoms such as fatigue,[28, 10] dull pain in the chest,[29] prolonged fever,[38] loss of weight,[29] and diarrhea,[38] have been recorded. Chaudhuri[7] reported that as many as 42 of 167 patients "had no pulmonary complaints, and were admitted for some other ailments, mostly gastrointestinal and the condition was detected accidentally." He also noted that some persons were entirely symptom-free. The diagnosis in these atypical cases was made on the basis of high eosinophilia and response to arsenotherapy. Of the 213 patients diagnosed as eosinophilic lung in the present study, 13 did not have the typical symptom of cough: one presented as a case of fever, four had chest pain and eight had no complaints. These eight had leukocyte counts done because routine chest films taken to exclude tuberculosis had revealed a radiological picture suggestive of eosinophilic lung. After treatment with diethylcarbamazine, all 13 patients showed a fall in the eosinophil count and relief of symptoms, where present. Their main clinical features and laboratory findings are given in Table 4. The only constant feature in these patients when first seen was the high eosinophilia, but this together with the positive FCFT and favorable hematological response to treatment justifies the conclusion that these are atypical cases of eosinophilic lung. The FCFT is, therefore, of value in the diagnosis of atypical cases, and its use would help in the elucidation of obscure cases of hypereosinophilia.

Spontaneous remission of symptoms with a decrease in the number of circulating eosinophils has been noted by various workers[40, 49, 10] in some patients diagnosed as eosinophilic lung; as they did not receive treatment with organic arsenicals, there was no confirmation of this diagnosis. The FCFT was carried out serially in four patients with cough and hypereosinophilia who showed spontaneous regression of symptoms and a fall in their eosinophil counts before any specific treatment was given: initial titers of 1:20, 1:40, 1:20 and 1:40, respectively, were obtained. These titers gradually diminished and became negative. The application of this test would, therefore, support the diagnosis in such cases where spontaneous remission occurs.

Etiology. The CF reaction using *D. immitis* antigen has been used by various investigators[16, 33, 5, 24, 50, 37, 42] in the diagnosis of human filariasis.

TABLE 4

Atypical cases of eosinophilic lung

Case No.	Symptoms	Eosinophils per cu. mm	FCFT titer
13	CP*	7,680	1/10
26	CP	12,296	1/20
31	CP	8,160	1/20
56	—†	23,328	1/320
62	CP	6,300	1/320
70	—	13,440	1/80
79	FE	22,445	1/320
118	—	23,360	1/5
125	—	8,446	1/40
147	—	8,400	1/40
152	—	5,600	1/20
153	—	16,484	1/160
157	—	4,785	1/40

* CP = chest pain; FE = fever.
† — = none.

According to Ridley,[43] the highest incidence of positive FCFT results was obtained in cases of loaiasis (85%), whereas only 24% of known *Wuchereria bancrofti* sera were positive. Minning and McFadzean[37] found that of 51 persons with microfilariae of *W. bancrofti* in their blood, only 57% were positive. They suggested the following possible explanation for the negative reactions in the remaining 43% of their cases: (a) that as the antigen employed was not homologous, optimal reaction with the antibodies present was not obtained in some cases; (b) that free antibodies might be absent periodically from the blood if they were adsorbed onto the microfilariae, or neutralized by an antigenic fluid from the adult worms, or if metabolic products of the parasites damaged the reticuloendothelial system; (c) that as the parasites are well adapted to their host, there is a poor antibody response. The results (see Table 5) obtained in the present study show the low incidence of positive CF tests with sera of bancroftian filariasis patients and confirm the findings of Ridley and of Minning and McFadzean. A similar low incidence was found in the sera of patients with filariasis due to *Wuchereria malayi* and in dogs infected with *D. immitis*.

The question posed as a result of the present investigation is whether the positive FCFT results obtained in cases of eosinophilic lung indicate an infection with a human filaria. Such an etiology has been previously advanced for eosinophilic lung on the basis of the histological findings of microfilariae (identified in some instances as *W. malayi*) in the lymph nodes of a few patients with hypereosinophilia, lymphadenopathy and pulmonary symptoms.[36, 45, 46, 34, 42, 6, 20, 9, 17, 52, 22] Important differences, however, exist between these proved cases of filariasis and those described as eosinophilic lung in which no definite etiological agent has so far been demonstrated. Lymphadenopathy of the degree described in the above reports is not seen in eosinophilic lung, nor are any of the other classical clinical features of filariasis found, such as lymphangitis, adenitis or elephantiasis, even in long-standing cases.

Inguinal lymph nodes are not uncommonly palpable in adults in the tropics and result from septic sores subsequent to minor injuries to the feet and legs of those who do not wear shoes. It is, therefore, not uncommon to find palpable inguinal lymph nodes in some cases of eosinophilic lung, but these are small. Extensive serial sections of palpable inguinal lymph nodes excised from four patients diagnosed as eosinophilic lung and demonstrating positive reactions to the FCFT did not reveal microfilariae, adult worms, or a histological picture suggestive of filariasis. On the other hand, in three patients who presented with "lumps" in the groins, but without either pulmonary symptoms or hypereosinophilia, histological examination of the excised lymph nodes revealed an adult filarial worm in one case and a histological picture suggestive of filariasis in the other two. Peripheral blood examination was positive for *W. bancrofti* in the patient with the adult filarial worm, but the FCFT was negative. In the remaining two patients no microfilariae were found in the blood, but the FCFT was positive (1:20 titer) in one of them.

TABLE 5

FCFT in human filariasis (Group 3)

Evidence of filariasis	No. of cases	No. with + FCFT	% positive
W. bancrofti microfilaremia only	35	5	14.3
W. bancrofti microfilaremia with clinical signs of filariasis	20	3	15.0
W. malayi microfilaremia only	34	2	5.9
Clinical filariasis	30	14	46.7
Totals	119	24	20.2

Furthermore, blood examinations for microfilariae in patients suffering from eosinophilic lung have been consistently negative. Of a group of 150 patients reviewed by Danaraj,[10] nocturnal blood films for microfilariae examined six to seven times in each case were negative in all 61 cases examined. Using Knott's concentration technique, 323 cases of eosinophilic lung have since been examined for microfilariae with negative results; the sputa of none of these patients revealed any parasites.

Conversely, hypereosinophilia is not a usual feature of filariasis. Of a group of 137 patients (including 85 patients from study Group 3) with filariasis diagnosed parasitologically or clinically, 84 had eosinophilia; of these only one had hypereosinophilia of more than 3,000 cells per cu. mm. This patient, whose count was 22,500 cells per cu. mm, had a mild diurnal cough of a few days' duration which cleared without any treatment.

Lymphadenopathy and other signs of filariasis were lacking, and the diagnosis was made on the presence of microfilaremia (*W. bancrofti*). The FCFT was positive in a titer of 1:10. The pulmonary radiographs were normal, and sputum examination showed no microfilariae. The eosinophil count decreased to 2,100 cells per cu. mm after treatment with diethylcarbamazine, and the FCFT became negative. Whether the cough was related to the filarial infection or was merely coincidental is difficult to say. However, pulmonary symptoms of the nature of Loeffler's syndrome have been described by Malhotra[34, 35] in patients with microfilaremia or other features of filariasis, and Rifkin and Eberhard[46] have reported one patient with pulmonary symptoms and eosinophilia in whom numerous microfilariae of *W. bancrofti* were found in the peripheral blood as well as in the sputum.

It is maintained that cases of eosinophilic lung and those of human filariasis present reasonably distinct clinical differences, and the presence of hypereosinophilia in an occasional case with demonstrable microfilariae or adult filarial worms does not necessarily indicate an etiological relationship between the two diseases.

Since a group-specific antigen is used, the FCFT is essentially a group reaction, the scope of which presumably includes the majority of the species of the superfamily Filarioidea. Therefore, the possibility of zoonotic filariasis occurring in man must be considered. As filarial worms are common parasites of frogs, lizards, birds and mammals throughout the world, and the vectors, where known, are blood-sucking arthropods, there exists the possibility that man may be infected with larvae of nonhuman filariae which, in view of their host specificity, do not mature in human beings. Augustine and Lherisson[1] explained the positive skin reactions following the use of *D. immitis* antigen in individuals without collateral evidence of filarial infection as being due to nonhuman filarial worms producing sensitization in man. Minning and McFadzean[37] showed that of 159 persons without microfilaremia living in an endemic area of filariasis, 33% gave a positive reaction to the FCFT. Although they concluded that human filarial infection was the probable cause of the serological reaction, they also mentioned that "theoretically animal filariae might be responsible for a positive reaction in a number of these cases." Human beings can rarely be infected by filarial worms of the genus *Dirofilaria*,[19, 18] but in no instance has there been found in man a fully mature *Dirofilaria* infection with microfilariae in the blood.[3] Thus, animal filariae, for which man is not the definitive host and which therefore do not mature sexually so that there is no microfilaremia, may still provoke group-specific antibody reactions in man.

Host specificity for filarial worms, as evidenced by the inability of larvae to attain sexual maturity in a "foreign" host, has been shown by Innes and Shoho.[26, 27] They reported that *Setaria digitata* which is usually found living harmlessly in the abdominal cavity of cattle, its normal host, is transmissible by mosquitoes to horses, goats, and sheep. In these abnormal hosts the immature worms do not develop into adults, but migrate at times to the central nervous system where they produce areas of malacia with resultant acute or subacute paralysis, sometimes ending fatally. The possibility of a similar type of infection occurring in human beings with damage to the brain and spinal cord was stressed by these authors, who felt that if the disorder was not fatal the clinical diagnosis would be impossible in the absence of skin or serological tests. Shoho[47] used diethylcarbamazine successfully in the treatment of this type of paralysis in sheep.

A further example of invasion of human tissue by a nematode normally parasitic in lower animals is visceral larva migrans[4] caused by infective-stage larvae of dog and cat ascarids of the genus *Toxocara*. The disease which occurs in young children has a variable symptomatology, but is characterized by chronic extreme eosinophilia (similar to that seen in eosinophilic lung) and granulomatous lesions in an enlarged liver. That extensive migration of the larvae occurs in human tissues is shown in a report of a necropsy in a child in whom granulomata, with and without larvae of *T. canis*, were found widely scattered throughout the viscera and central nervous system.[15] However, where larval migration is not extensive, but confined chiefly to the skin, as in cases of cutaneous larva migrans caused presumably by larvae of cat and dog hookworms, a similar hypereosinophilia has not been noted. The migratory pulmonary shadows seen in the radiographs of some of these cases are thought to be allergic in nature similar to Loeffler's syndrome.[50] The etiological diagnosis in both types of larval migrans is difficult as this depends on

the demonstration of larvae in tissue obtained by biopsy or at post-mortem; serological tests may soon be available.[55, 39]

Where parasites and hosts are well adapted to each other, there does not appear to be much tissue response, as was demonstrated by the low titers and often negative FCFT reactions in sera of patients with human filariasis and dogs infected with *D. immitis*. In cases of eosinophilic lung, however, the massive eosinophilic leukocytosis and high level of production of antibodies demonstrated by the FCFT suggest a severe tissue response such as might be expected in infections where the host-parasite relationship is abnormal.

The finding of sensitivity to filarial proteins in patients suffering from eosinophilic lung, although not definitely establishing an etiology, assists by pointing to the superfamily Filarioidea, as do results of treatment with diethylcarbamazine, an antifilarial drug. The three possibilities to be considered are: (1) an alteration of host immunity to some human filarial infections giving rise to allergic phenomena manifested by eosinophilia and pulmonary symptoms; (2) abnormal migratory routes and localization sites of human filaria within the host; and (3) infection by animal filariae normally not pathogenic to man.

It is thus possible that eosinophilic lung is a manifestation of filariasis which has not been described previously due to the absence of microfilaremia at any stage. If this hypothesis is true, eosinophilic lung may be prevalent, although not diagnosed as such, in wide areas of the world where filariasis is endemic. Although the geographical distribution of eosinophilic lung is not fully known, reports of the disease in the literature so far tend to show it existing in areas known to be those of endemic filariasis.

SUMMARY

The complement fixation test using as antigen a 1% alcoholic extract of dried *D. immitis* powder was applied to the sera of: 228 persons with hypereosinophilia of 3,000 or more cells per cu. mm, 213 of whom were diagnosed as eosinophilic lung; 85 persons with mild eosinophilia of less than 3,000 cells per cu.mm; 119 cases of human filariasis; 33 dogs with *D. immitis* infection; 30 cases of severe ascariasis; 160 normal individuals. The test was positive, in the majority of instances in high titer, in 95.3% of the cases of eosinophilic lung, whereas in the various control groups, positive reactions in relatively low titers were obtained in only 5.6% to 21.2% of cases. After treatment with diethylcarbamazine, the FCFT titers gradually diminished in all the patients suffering from eosinophilic lung, becoming negative in 95.4% of those who had completed the full observation period of 30 weeks.

It is felt that the FCFT can be used to differentiate eosinophilic lung from other conditions associated with an eosinophilia. The value of the test in the diagnosis of atypical cases is shown.

In view of the finding of sensitivity to filarial proteins in cases of eosinophilic lung, the etiological possibility of a filarial infection, not necessarily human, is discussed.

ACKNOWLEDGMENTS

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ADDENDUM

Since this paper was written, support for the hypothesis that eosinophilic lung may be due to infection with animal filariae comes from Buckley[56] who was able on two occasions to reproduce the disease in a human volunteer by inoculating him with filarial parasites obtained from mosquitoes fed upon a monkey and a cat, respectively. The patient developed cough, hypereosinophilia and a strongly positive reaction to the FCFT. Treatment with diethylcarbamazine resulted in an amelioration of symptoms which was associated with a striking fall in the number of circulating eosinophils; the FCFT, which was positive in a titer of 1:640, declined to 1:20. The experiment failed to produce a mature filarial infection in the volunteer as no microfilariae appeared in the blood.

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OBSERVATIONS ON *ANOPHELES (KERTEZIA) BELLATOR* D. & K. IN BRITISH GUIANA*

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Overseas Civil Service

In his comprehensive review of the *Kertezia* anopheles Komp[21] critically examined the previous literature on, and described seven valid species of, this subgenus. They were characterized by a breeding habitat limited to the water-holding leaf "tank" at the base of bromeliad plants or, in the case of *A. anoplus*, to bamboo joints. As a group, they were confined in their geographic distribution to South and Central America and Trinidad.

Anopheles bellator was confirmed by Komp only from its type locality in Trinidad, West Indies, and Brazil, since he was unable to identify specifically specimens of *Kertezia* available to him from Venezuela, while those reported as *A. bellator* from Dutch Guiana by Bonne and Bonne-Webster[4] were thought to be *A. anoplus*. A later monograph by Cova-Garcia[6] established the presence of *A. bellator* and three other *Kertezia* species in Venezuela.

Although Russell, Rozeboom and Stone[25] accepted Komp's[21] description of *A. anoplus* and included it in their keys, this species was not retained among the subgenus *Kertezia* by Lane[22] who considered *A. anoplus* to be identical with *A. homunculus*. Lane,[22] however, listed both *A. neivai* and *A. cruzii* as occurring in the Guianas. The presence of *A. neivai* only had been reported from French Guiana by Floch and Abonnec,[15] and no recent record of *A. cruzii* collections from this general area could be traced. Lane's reference was apparently based on Dyar's[11] use of *A. cruzii* as a synonym for *A. neivai* in Surinam.

In any event, no reference to British Guiana was encountered in the literature on the *Kertezia* anopheles. It is the purpose of this paper to record the presence of *A. bellator* in British Guiana, and outline the results of surveys on its local distribution and probable role as a secondary vector of malaria.

METHODS

Searches for larvae were first made in arboreal bromeliad plants harboring in the forest in an area in the North West District of British Guiana (see map) where the presence of *Kertezia* anopheles appeared likely from the results of a routine adult capture. Subsequent surveys carried out over a wide range of the hinterland areas of the Colony were based primarily on a search for the larger types of bromeliads made during journeys in an outboard motor launch on the rivers, or on treks along forest trails. In those localities where such bromeliads were seen, a representative number of the various species present was examined as described below.

Where the plants were readily accessible, their water content was emptied directly into a white enamel basin, but where more than moderate climbing was necessary the contents were lowered by bucket and rope and transferred to basins at ground level. After manual removal of the larger floating debris, the water was allowed to stand for approximately 10 minutes before examination. Larvae and pupae were collected with a pipette, and those surviving the journey to the laboratory were reared in water taken from bromeliad plants. Dried brewers' yeast was used as larval food.

Adult mosquito captures were made off human bait in the evenings immediately after the onset of dusk, either at ground level in the open forest or in the motor launch moored alongside the river banks.

Malariometric surveys, limited to indigenous Amerindian children, comprised palpation for splenic enlargement with the subject lying on the ground, and examination of thick blood films stained with Giemsa.

Bromeliad plants were either identified for species by the Department of Agriculture in

* The observations recorded in this paper were carried out partly in 1952–1954 during a tour of Service in British Guiana and partly in 1958 under a research grant awarded by the Standing Advisory Committee for Medical Research in the British Caribbean Territories.

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British Guiana, or were prepared for shipment to the Kew Gardens Herbarium for verification.

RESULTS

Geographical distribution of A. bellator *in British Guiana*

In September, 1952, during routine mosquito captures made by the Mosquito Control Service's riverain DDT-spraying squad,[2] Mr. A S. Clarke, Operator-in-Charge, collected three female adult anopheles at Koriabo, Barima River, in the North West District of British Guiana. The specimens were small, and although they reached the laboratory in a battered and moldy condition, they were tentatively classified by the author as members of the subgenus *Kertezia*.

In July, 1953, during a malaria control sortie in the headwaters of the Barama River, one *A. bellator* female was collected by the author during an evening capture at Lewis' Camp near Baramita, also in the North West District.

In May, 1954, these findings were further investigated, and larvae of *Anopheles bellator* were collected from the arboreal bromeliad *Guzmania capituligera* at Koriabo, Barima River. Male and female adults and a number of fourth instar larvae reared from this collection were confirmed as *A. bellator* by Dr. Alan Stone (personal communication) but a single larva of the subgenus *Kertezia* having simple post-spiracular hairs could not be identified as to species. Subsequent investigations during 1954 revealed the presence of *A. bellator* along the upper reaches of the Potaro River, and on the Kaieteur escarpment.

A more comprehensive survey during January–February, 1958, added as localities for *A. bellator* only the other major rivers of the North West District, viz., Waini, Kaituma and Aruka Rivers, and an apparently independent focus on the east bank of the Essequibo River at Rockstone (Fig. 1).

Rainfall and A. bellator *distribution*

The general distribution of rainfall in British Guiana, shown in Fig. 1, was consolidated from data supplied by the Department of Agriculture.

The areas of *A. bellator* encountered in the course of the surveys described in this report were all within the northern two thirds of the central belt of heavy rains, with the foci in the North West District and Essequibo River lying within the 91 to 120 inch zone, and the Potaro-Kaieteur focus extending partly into the rainfall belt of over 120 inches. It must be added, however, that detailed observations were not made in the north-western section of this zone of heaviest rainfall which includes the Wenamu-Upper Mazaruni-Mt. Roraima areas. Furthermore, only limited searches were made on the Rupunnuni River and in the southern rainfall zone of 91 to 120 inches.

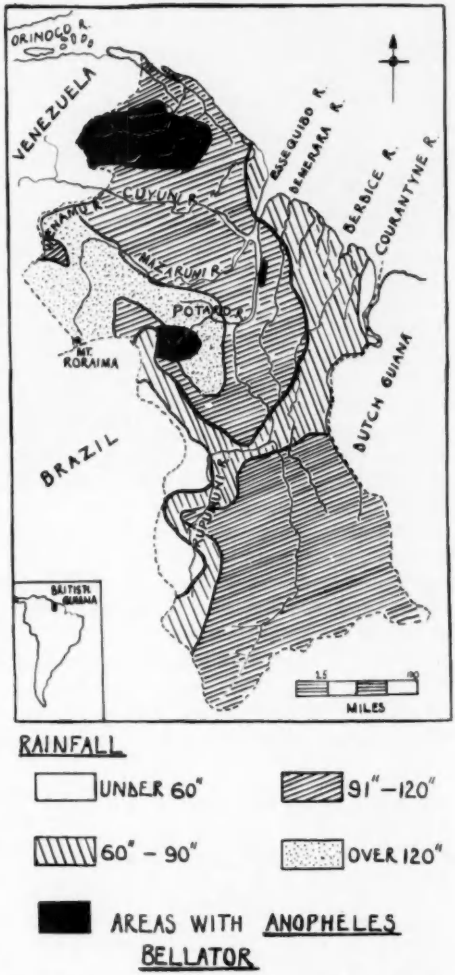

FIG. 1. Showing distribution of rainfall and *Anopheles bellator* in British Guiana.

The bromeliad flora and A. bellator

Prior to 1954 Smith[26] had listed 51 species of bromeliads occurring in British Guiana, and had suggested five additional species likely to be found. From collections made in 1944, however, Smith[27] described seven other species, while among the plants submitted by us to the Kew Gardens Herbarium, three species new to British Guiana were recorded (Salisbury, 1955, personal communication), including one of Smith's probables. At the present time, therefore, a total of 61 bromeliad species are known to occur in the Colony. Of this total, *Aechmea nudicaulis* was the most common, and occurred in high density on the coastlands, along the lower tidal reaches of the rivers, and in the forests of the hinterland. With the exceptions mentioned hereunder, however, the other bromeliad species encountered were scanty in number, usually of limited regional distribution, and occurred only under natural forest conditions. Five arboreal and one terrestrial species were found serving as host plants to *A. bellator*. These are briefly discussed below, and illustrated in Figures 2 and 3 with their characteristic inflorescence.

Guzmania capituligera Gresb was restricted to the river systems of the North West District, beyond the upstream limits of brackish tidal waters and the associated *Avicennia* mangroves. The epiphyte showed no apparent predilection for the numerous giant species which constituted the typical rain forests in this area, but it attained maximum density in a 25-mile section of the Barima River basin, centered at Koriabo Creek 75 miles from the coast. Here, the dominant forest trees were Mora (*Mora excelsa*) and Water Wallaba (*Sarabebe macrolobium*), individual members of which harbored upwards of 200 *G. capituligera* at top- and sub-canopy levels. On smaller trees, however, *e.g.*, Wild Fig (*Ficus sp.*) and "Iron Mary" (*Licania sp.*) the epiphyte commonly occurred within 4 to 6 feet of ground level, especially along the river banks.

Both during the wet month of May, 1954, and in the dry season of January, 1958, 80% of fully-grown plants of this species were positive for *A. bellator* at a density of 1.25 fourth-instar larvae per plant. No appreciable difference between frequencies or densities of anopheles larval infestations was observed at varying vertical levels in the forest.

Vriesia platynema Gaud., and less commonly, *Vriesia albiflora* Ule were encountered only at Koriabo, Barima River. *V. albiflora* was more scanty in its distribution, usually interspersed among *V. platynema*, and it supported *A. bellator* breeding to the same extent as the latter, 60% being positive with seldom more than one fourth-instar larva per positive plant

FIG. 2. Showing three principal *A. bellator* host plants in British Guiana: (a) *Guzmania capituligera*, (b) *G. Altsonii*, (c) *Brocchinia micrantha*.

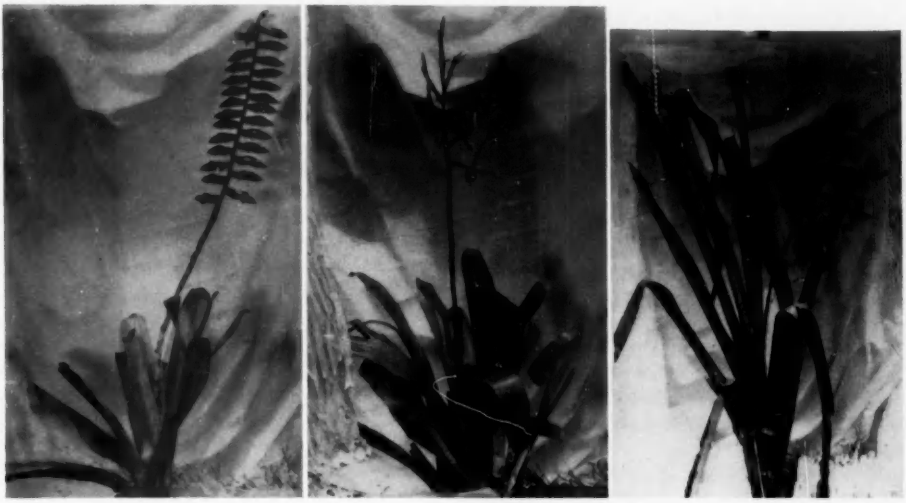

FIG. 3. Showing three additional *A. bellator* host plants in British Guiana: (d) *Vriesia platynema*, (e) *V. albiflora*, and (f) *Wittmackia odora*.

Wittmackia odora (Miq.) Mez was observed at low density in association with the above two *Vriesia* species at Koriabo where 60% of the specimens examined were positive for *A. bellator*. In the Kaituma River this bromeliad was also present in small numbers from the Anabisi Creek upwards, but during January, 1958, the water content of the plants was small, and no *A. bellator* breeding was found in 20 specimens examined. At Rockstone, on the east bank of the Essequibo River, however, *W. odora* was found in large numbers on a mango and a eucalyptus tree with a 30% *A. bellator* infestation rate.

Guzmania Altsonii Smith was limited to the Potaro River and its Konawaruk tributary, and the Kaieteur escarpment. Like that of *G. capituligera* in the North West District, its vertical distribution ranged from top canopy to near ground levels. On the Kaieteur escarpment it was not uncommonly found as a terrestrial plant growing both on rocky outcrops and on virgin soil, supporting *A. bellator* breeding in all locations.

Brocchinia micrantha (Bak.) Mez, a large terrestrial bromeliad, was indigenous to the rocky escarpment formations which characterize the central plateau of British Guiana. On the top of the Kaieteur escarpment it grew profusely in large fields, and individual plants attained heights of 6 to 10 feet before blooming an 8- to 20-foot inflorescence.* Even under the dry conditions of January, 1958, 4 gallons of water between the leaf axils of a large plant was not an unusual finding, with 12 or more fourth-instar larvae of *A. bellator*. All ten plants examined were positive.

Three other bromeliads were encountered which, from their general resemblance in form and size to the major arboreal host plants described, appeared suitable for *Kertezia* breeding, viz., *Vriesia procera* in the Barima River, and *Guzmania cryptantha* and *G. lingulata* at Kaieteur. They were present in only small numbers, however, and the few specimens available for examination were free from anopheles larvae. Two of these species (*V. procera* and *G. lingulata*) were recorded as important *A. bellator* breeding plants in Trinidad.[9] But *Gravisia aquilega*, reported by these two authors as the dominant host plant in the cocoa forests of Trinidad, was found only once in British Guiana in a suburb of New Amsterdam on the eastern coastlands.

* The illustration (Figure 2) of this plant with full inflorescence is reproduced by kind permission of the Editor, *Gardner's Chronicle*, where it appeared in 1900, ser. 3, vol. 28, p. 185 under the name *Brocchinia cordylinoides*.

A. bellator *adult density*

The results are summarized, in Table 1, of a small number of evening, human-bait, adult mosquito captures made, once on each occasion, at the more important *A. bellator* breeding localities. *A. bellator* adult density was approximately one per man-hour during both dry and wet seasons, but at Konawaruk airstrip, Potaro District, and at the Kaituma River none was captured in the single observations made in 1954 and 1958, respectively.

At Kaieteur Falls, however, in a small patch of open savannah with a heavy growth of *Brocchinia micrantha* nearby, 180 adult *A. bellator* were taken off an assistant in January, 1958. These anopheles attacked furiously with the first onset of dusk, concentrating on the lower limbs. They attained a peak density at 6.30 p.m., and by 7.15 p.m. when collection was discontinued, their number had been drastically reduced.

A. bellator *and malaria in British Guiana*

In British Guiana, the malaria control program directed against the principal vector *A. darlingi* was successful both on the densely populated coastlands and in the isolated settlements scattered in the hinterland.[16, 17] In January, 1951, a mobile spraying team was established to extend this operation on an annual cycle to the riverain areas.[2] This produced equally good results, and *Anopheles darlingi* invariably disappeared in these localities after the first DDT applications. In a field experiment on the coastlands under very poor conditions of housing and domestic cleanliness Giglioli[18] failed in deliberate attempts to encourage the re-establishment of *A. darlingi* (and *Aedes aegypti*) within the perimeter of a group of houses previously subjected to six DDT spraying cycles in 4 years. Since 1952, such small and new pockets of endemic malaria as came to light were associated with *A. darlingi* either in hitherto unsprayed localities,[3, 4] or in border areas where the spraying schedule had not been adequately maintained.[5] Because of these considerations, *A. bellator* was viewed as a probable vector in Koriabo, where this anopheles was the only species present, and where signs of malaria transmission were evident.

In a general health survey of the British Guiana hinterland, Eddey[12] noted, in 1947, a widespread picture of chronic malaria on the upper Barima River, "with spleen rates of 40 to 80 per cent for all age groups in the various settlements." The report did not show details for each settlement, but the data quoted included those for Koriabo.

In 1949 the first residual DDT sprayings of the upper Barima were carried out and from 1951 they were undertaken annually by the Mosquito Control Service's riverain squad.

Results of malariometric surveys made periodically by the author on all the Amerindian children resident at Koriabo are summarized in Table 2. The spleen rate fell from 100% in 1951 to zero by 1954, but in 1958 this rate had risen again to 11.5%. During the period of observation, however, the parasite rate apparently remained unchanged at approximately 12%. The majority of the spleens palpable in 1951 were tough, fibrotic, palpable in, or posterior to, the mid-axillary line, and appeared to represent old, enlarged spleens undergoing resolution. In

TABLE 1

Results of single evening human-bait captures for A. bellator *adults in British Guiana*

Date of survey	Locality	*A. bellator* density per man hour
Sept. 1952	Koriabo, Barima River	1.50
July 1953	Lewis Camp, Baramita	1.00
May 1954	Koriabo, Barima River	1.00
Aug. 1954	Konawaruk Potaro R.	—
Jan. 1958	Koriabo, Barima River	0.66
Jan. 1958	Anabisi, Kaituma River	—
Jan. 1958	Kaieteur Falls	180.00

TABLE 2

Results of malariometric surveys on all indigenous Amerindian children resident at Koriabo, Barima River, British Guiana

Date	Total examined*	Spleen rate (%)	AES†	Parasite rate (%)
Aug. 1951	8	100.0	2.50	12.5
May 1954	14	—	—	14.3
Jan. 1958	26	11.5	1.66	11.5

* Because of the small numbers, age groups are not shown, but each sample comprised infants under 1 year and children aged 1 to 10 years.

† AES = average enlarged spleen.

contrast, the few enlarged spleens found in 1958 were soft, elastic, and extended below the costal margin in the mid-clavicular line; they resembled more closely the enlarged spleens associated with recent malarial infection

All the three positive blood slides encountered in 1951, and in 1954, contained *P. falciparum*, while the three found in 1958 were positive for *P. vivax*. One of the latter was from an infant 1 year old whose spleen was not palpably enlarged, while another occurred in a 3-year-old child with a size-2 spleen.[19]

In 1957 approximately 360 blood slides were examined for malaria in British Guiana, of which only three were positive. One of these, recorded by the Central Medical Laboratory, was from a resident of Koriabo, Barima River.

These epidemiological observations all indicated the presence of recent and active malaria transmission in this locality.

DISCUSSION

In his monograph on the anopheles of Venezuela, Cova-Garcia[6] listed four species of the subgenus *Kertezia*, with *A. bellator*, *A. boliviensis*, and *A. cruzii* in the Teritorio Amacuro and *A. homonculus* in the State of Bolivar. Both these administrative divisions of Venezuela abut with the North West District of British Guiana from which they are separated only by the Amakura River. It would therefore appear that the *A. bellator* focus recorded in this section of British Guiana was continuous with that of north-eastern Venezuela.

Since Bonne and Bonne-Webster's record[1] of *A. bellator* in Dutch Guiana (Surinam) has not been confirmed either by Komp[1] or Lane,[22] and since Fauran (1958, personal communication) reports that only *A. neivai* of the *Kertezia* anopheles has been found in French Guiana, the results of the present survey appear to establish British Guiana as the eastern limits of *A. bellator* distribution along the north coast of the South American continent.

Apart from Komp's reference[21] to the wild pineapple plant *Ananas magdalenae* as a breeding focus for *A. neivai* in Panama, *B. micrantha* in British Guiana appears to be the only other terrestrial bromeliad involved in the *Kertezia* complex, although Davis[7] recorded that some of the Brazilian host plants grew both on forest trees and on rocks. This was also observed here for *G. Altsonii* at the Kaieteur escarpment.

Final incrimination of *A. bellator* as a vector of malaria under conditions existing in British Guiana presents a formidable problem largely because of low adult *A. bellator* densities occurring in sparsely inhabited areas where the parasite reservoir in the indigenous population has been greatly reduced by the successful DDT residual spraying campaign directed against *A. darlingi*. For, even in the cocoa-growing areas of central Trinidad where high malaria incidence was associated by de Verteuil[8] with large swarms of *A. bellator* adults, conclusive proof of its vectorial role was obtained only in three infected specimens of 725 examined by Rozeboom and Laird.[24] And Downs and Pittendrigh[10] have summarized only 14 positive findings among 2,932 wild-caught *A. bellator* dissections reported in the literature.

The epidemiological evidence presented in Table 2 for the small community at Koriabo, Barima River, however, is strongly indicative that here, at least, *A. bellator* is actively engaged in the transmission of malaria in British Guiana, albeit on a small scale. The high spleen rate associated with low parasite rate in 1951 clearly reflected incomplete resolution of fibrotic spleens after control in 1949–50 of *A. darlingi* and the general situation of widespread malaria recorded by Eddey[12] for the upper Barima River. These old, enlarged, malarial spleens had receded to normal size by 1954, but indications of fresh infection in children aged 1 to 10 years were evident both from the splenic enlargement and positive parasitemia recorded in 1958. Since the locality had been subjected to routine annual "barrier" DDT residual spraying from 1949 onwards, and since *A. bellator* was the only anopheles still being collected there (Table 1), it was difficult to escape the conclusion that this mosquito was the responsible vector.

The probable role of intransit motor-launch travellers serving as the source for this fresh infection, or as the disseminators further up river of a focal infection indigenous to Koriabo, is a consideration of prime importance to proposals under review for implementing the newer policy of malaria eradication in British Guiana. It is known that immigrant labor is recruited from the Eastern Caribbean. In those islands the status of malaria control remains generally less satisfactory than that already achieved in British Guiana,[23] and attention was directed to

this danger of immigrant sources of infection by the W.H.O.[28] Indeed, in the neighboring territory of French Guiana, Floch[13] already has had to deal with this problem when, in 1954, he recorded several hundred cases of malaria occurring among immigrants from the island of St. Lucia, and in the local residents of the area where they had settled. While advocating the adoption of quarantine measures against malaria, Floch[14] instituted routine suppressive treatment of suspected immigrants.

In British Guiana, therefore, quite apart from the small settlement at Koriabo where evidence of transmission was uncovered, the potential threat of *A. bellator* malaria presents itself in all the areas infested by this anopheles where development projects may attract infected personnel or visitors.

Of particular significance in this connection was the coincidence of the suitability of lands for cocoa cultivation in the *A. bellator* strongholds in the North West District.[20] For, although *A. bellator* now occurs there only at comparatively low density, the experience in Trinidad cannot be overlooked where forest clearance, followed by successful establishment of cocoa plantations shaded with immortelles (*Erythrina spp.*), unfortunately provided conditions which "proved to be also particularly favourable to the growth of water-holding bromeliads especially on the immortelle shade trees."[8] These changes in the ecological conditions were soon followed by a fabulous increase in *A. bellator* density and an associated high malaria prevalence.

These potential malaria problems in the British Guiana hinterlands may well be nullified by the concerted efforts at eradication now being stimulated in the Americas by the Pan-American Sanitary Bureau and World Health Organization. A successful result in these programs will eliminate the external threat of importation into British Guiana of human reservoirs of malaria from the Eastern Caribbean, and prevent dissemination from local foci like that described at Koriabo.

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ON THE CULTIVATION OF *TRYPANOSOMA BRUCEI* IN VITRO*

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Trypanosoma brucei was first successfully grown *in vitro* by Novy and MacNeal during 1904.[5, 6] This important accomplishment was confirmed by Behrens.[2] However, since 1916, many investigators, including the writer both in Prof. F. G. Novy's laboratory at Ann Arbor, Michigan, and elsewhere, have tried to grow this particular strain of *Tr. brucei* in vitro without success. These failures probably were due to the fact that the Novy strain of *Tr. brucei* after syringe passage in guinea pigs for nearly 30 years had lost the ability to grow in vitro. Brutsaert and Henrard[3, 4] stated that they were able to culture another strain of *Tr. brucei* on the media of von Razgha[15] consisting of citrated human blood and Ringer's and Tyrode's solutions.

The object of the present communication is to report successful cultivations in vitro of two Uganda strains of *Tr. brucei* from 74 experimentally infected animals.

METHODS AND MATERIALS

The strains of Trypanosoma brucei. Two African strains of *Tr. brucei* were used in this study. One of these strains, infective to domestic and laboratory animals but not to man, is known as Shinyanza III, and is kept in domestic animals through continuous transmission by tsetse flies (*Glossina morsitans*) at the Central Trypanosomiasis Research Laboratory at Uganda, Africa. In July, 1954, laboratory animals infected with this strain, received through the courtesy of Dr. M. A. Soltys and Dr. K. C. Willett at Tororo, Uganda, were brought to the United States by the writer.

The other strain of *Tr. brucei* known as Bussi was originally isolated from a dog on October 5, 1953, by C. Kyewalyanzo. The blood from the dog was inoculated to mice and the strain was kept in mice by continuous syringe passage, at the Veterinary Medical School, Entapee, Uganda. The 64th animal passage (6 August, 1954) was brought to the United States by the writer.

The above two strains of *Tr. brucei* on arrival at Galveston, Texas, were kept independently of each other in guinea pigs, rabbits and American deer mice (*Peromyscus maniculatus gambeli*). Since 1956 the stock of these strains of *Tr. brucei* have been kept in *P. m. gambeli*. Because the disease in this species runs a subacute course lasting from 60 to over 200 days this procedure is inexpensive and safe. Whenever one drop of tail blood from deer mice (*P. m. gambeli*) infected with *Tr. brucei* is inoculated to house mice (*Mus musculus*) or rats (*Rattus norvegicus*), it invariably produces infection which terminates fatally within 6 to 12 days.[9]

Culture media. The principal culture medium used for this study was a slight modification of Novy and MacNeal (NN) medium. The chief differences between this modified medium as compared with the original NN medium consist of the use of double-infusion beef meat (cold and hot extraction), the use of NaOH instead of Na_2CO_3 for adjusting pH, and the use of Difco peptone instead of Witte's peptone. For convenience, this so-called "modified medium" is referred to as NNP (Novy, MacNeal, and Packchanian) medium.

Beef infusion broth as indicated above containing 1.3 to 2% agar and from 25 to 75% defibrinated rabbit blood has been used since 1928 by the writer for cultivation of hemoflagellates and has been referred to in all earlier publications as NN medium.[7, 8, 10, 13, 14] However, since some investigators do not accept these slight changes as identical to NN, it is deemed desirable at this time to give a detailed description of the preparation of the modified NN medium.

Preparation of NNP Medium

This medium can be prepared in four independent stages as follows: the basic beef infusion broth, the combination of this infusion broth

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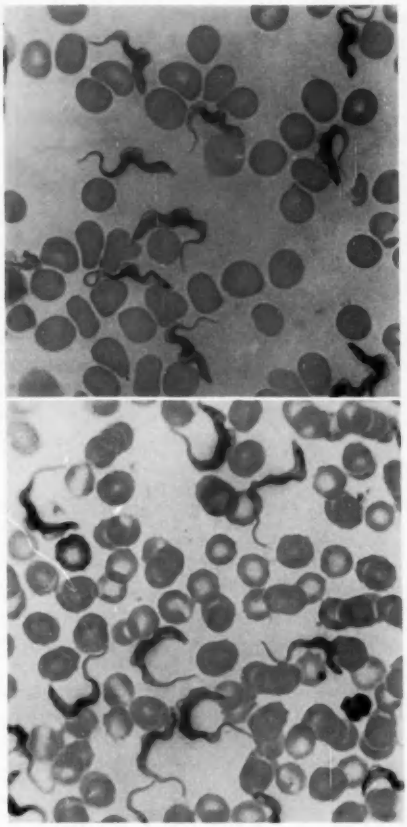

FIG. 1. *Trypanosoma brucei*, blood smear from a mouse, Giemsa stain (× 793).

with agar, the collection and defibrination of rabbit blood, and the mixing of the blood with the infusion agar to form the final medium.

(a) *Beef infusion broth.* To finely-ground, fresh, lean beef was added twice its weight of distilled water (500 g meat, 1000 ml dist. H_2O). These were thoroughly mixed and allowed to infuse (digest) overnight in the cold room (4°C). The following morning this mixture was "infused again" by heating gradually and boiling for about 10 minutes. The mixture was then strained through gauze. To this extract, while hot, was added 5 g NaCl and 10 g Difco Bacto-peptone. The reaction was adjusted to pH 7.8 with 1 N NaOH. It was then boiled an hour, filtered through filter paper until clear, volume was restored to 1000 ml with distilled water, and the infusion was autoclaved in a flask at 15 lbs. pressure for 45 to 60 minutes, and stored in a cold room or at room temperature until ready for use. (Usually a volume of 20 liters was prepared at a given time as stock infusion broth.)

(b) *Infusion agar.* To 1000 ml of infusion broth after filtering, 20 g of fine, powdered Difco Bacto-agar was added. After dissolving the agar by vigorous boiling for about 1 hour, volume was restored with distilled water, and the mixture was distributed to flasks (100 ml agar per 350-ml flask). This infusion (nutrient) agar was then labeled and autoclaved for 45 minutes at 15 lbs. pressure and stored in the cold room until ready for use. After autoclaving the pH usually dropped to about 7.4.

(c) *Rabbit blood.* About 50 ml of blood drawn by heart puncture from a healthy rabbit was introduced into a sterile flask containing glass beads and immediately defibrinated by vigorous shaking for about 5 minutes; the blood was then stored in a cold room for 1 to 3 days before use.

(d) *Final medium.* A flask containing 100 ml of 2% infusion agar was melted and allowed to cool in a 55°C water bath. To this melted agar was added 1, 2 or 3 volumes of warmed, defibrinated rabbit blood, agar and blood were mixed thoroughly, and the blood agar was dispensed with a bulb Pasteur pipette[12] to previously cooled test tubes (150 x 15 mm), 6 to 8 ml per tube, and slanted in a cool tray with 10-mm butt. After solidification, the tubes were placed in an upright position and stored in the cold room. The following day the tubes were brought to room temperature (25°C) and allowed to warm for a few hours to secure additional water of condensation. The tubes were then returned to the cold room until ready for use within a week or two following preparation. Occasionally smaller tubes (100 x 12 mm) containing 3 to 4 ml of medium were used.

The NNP medium usually contained 75% rabbit blood (1 part agar, 3 parts blood), or 66% blood (1 part agar, 2 parts blood). After a few subcultures of trypanosomes on NNP medium, the blood concentration of the medium was reduced to 50% (1 part agar, 1 part blood). While in most experiments whole rabbit blood was used, in some experiments serum was substituted for whole blood in the same percentage.

The Preparation of NNP-2 Medium. This medium, which contains less blood, is used chiefly for the growth of trypanosomes less fastidious than *Tr. brucei*. The preparation of NNP-2 medium is essentially the same as NNP, described above, except that to infusion broth was added only 1.3% agar instead of 2% and only 20 to 40% defibrinated rabbit blood instead of 50 to 75%. Since there is less blood in NNP-2, the agar percentage was reduced in order to obtain sufficient water of condensation in the tubes.

Experimental Animals. A total of 78 normal animals, consisting of 48 house mice (*Mus musculus*), two American deer mice (*Peromyscus maniculatus gambeli*), 24 rats (*Rattus norvegicus*) and four guinea pigs, were infected with *Tr. brucei*. At various stages of the disease when from two to over 200 trypanosomes per microscopic field (10 x 15) were present in the peripheral blood, the heart blood from these animals was removed under aseptic precautions and inoculated into culture medium.

Cultivation in vitro. For each cultural attempt, a minimum of six NNP tubes were inoculated with blood from infected animals. The amount of infected blood per tube varied from 0.1 to 0.5 ml. In some experiments, the blood drawn from the infected animal was immediately inoculated to NNP tubes; in others, the infected blood was first defibrinated and then inoculated to NNP tubes. All the tubes were rubber-capped and usually incubated at 25°C and occasionally at 30 or 37°C for comparative study. In some experiments several other types of media were used for initial cultivations and for subsequent subcultures for comparison with NNP medium; these consisted of original NN media, one of its modifications known as NNN (Novy, MacNeal and Nichols), NNP-2, Behrens dialyzed meat-extract blood agar and Behrens bean-extract blood agar.[2, 16]

The growth of Tr. brucei *in vitro.* One loop of material from the water of condensation of each tube was examined microscopically for growth of flagellates at the end of 7, 14 and 21 days of incubation. The positive cultures were carefully examined for extent of growth of flagellates with special reference to free-swimming, dividing forms and rosette formations.

Subcultures. Subcultures were made at intervals varying from 1 to 8 weeks. About 0.1 ml of the water of condensation from the tube showing the best growth was inoculated into two fresh NNP tubes, which were rubber-capped and incubated usually at 25°C.

Morphologic and biologic characteristics of cultural forms of Tr. brucei. The morphology of *Tr. brucei* both from initial culture and subsequent subcultures was studied by direct microscopic examination and by staining procedures. Smears from cultures were fixed with methyl alcohol and stained by Novy's modification of Romanovsky and by Giemsa stains.

Infectivity of cultures to animals. Forty-eight animals were used for testing infectivity of *Tr. brucei* cultures. Of these, 19 mice were treated prior to inoculation in order to reduce their resistance: 11 being exposed to 450 r x-ray radiations 5 to 12 days before testing and eight being injected with aminopterin 1 mg/kg intraperitoneally for 2 successive days 24 days prior to inoculation with cultures. The remaining 25 mice and four rats were normal. The entire growth of flagellates from one or two tubes was inoculated into each of these animals. The tail blood of the test animals was examined frequently for evidence of *Tr. brucei* infection, and they were kept under observation 2 to 3 months before they were discarded as negative. At the termination of the experiment the heart blood from some of these "negative" animals was inoculated to NNP tubes for possible evidence of flagellates.

EXPERIMENTAL DATA

Both Shinyanza III and Bussi strains of *Tr. brucei* grew readily on NN and NNP media from 74 experimentally infected animals regardless of whether the inoculum came directly from infected rats (*Rattus norvegicus*), house mice (*Mus musculus*), American deer mice (*Peromyscus maniculatus gambeli*), or guinea pigs. These findings can be described briefly as follows:

Heart blood from 47 *Mus musculus*, 21 infected with Shinyanza III strain and 26 with Bussi strain at various stages of the disease, all yielded positive cultures of flagellates. One negative result was obtained with a *Mus musculus* infected with Shinyanza III strain. Two *Peromyscus maniculatus gambeli* infected with the Bussi strain yielded one positive and one negative result. Ten rats infected with Shinyanza III strain and 14 with the Bussi strain all yielded

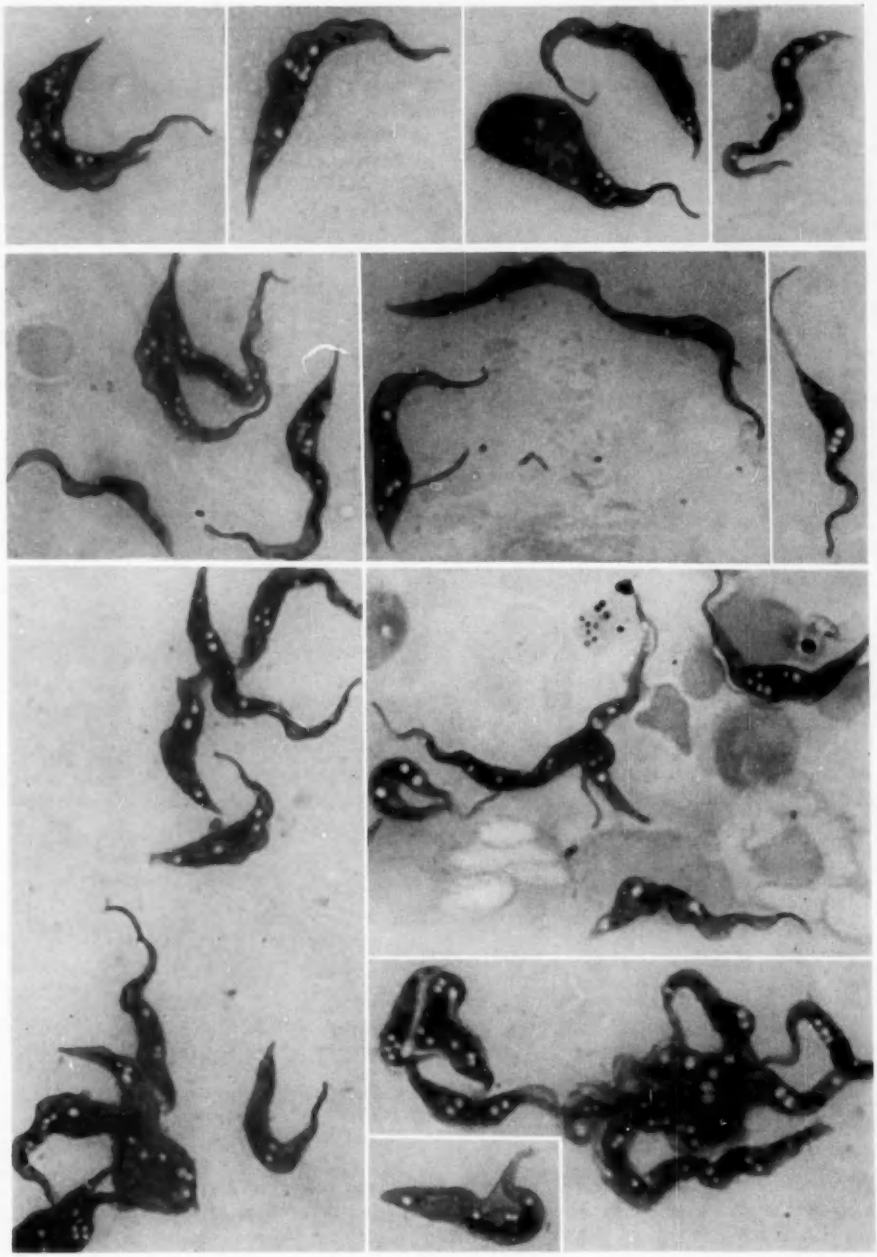

FIG. 2. *Trypanosoma brucei*, cultural forms, Giemsa stains (× 1170).

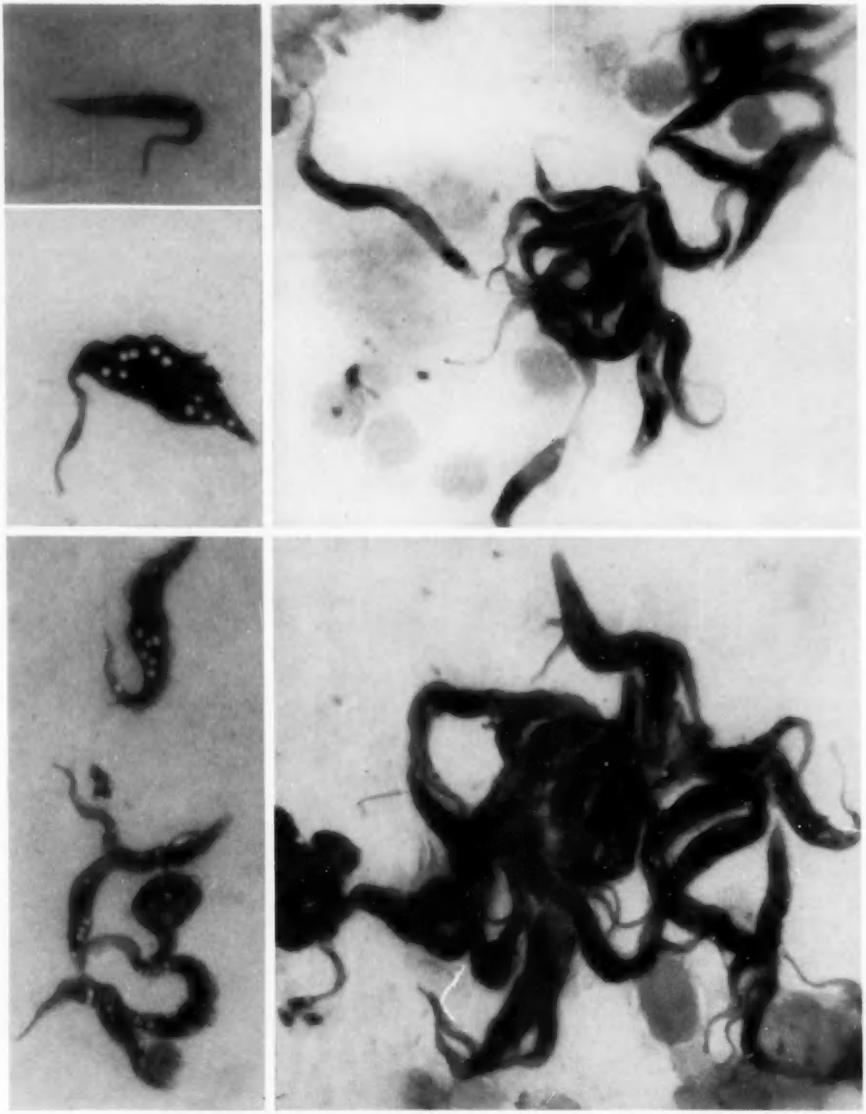

FIG. 3. *Trypanosoma brucei*, cultural forms, Giemsa stain (× 1425)

positive cultures. Positive results were obtained in two of four guinea pigs infected with Shinyanza III strain.

Growth of *Tr. brucei* occurred on NNP medium with pH ranging from 6.8 to 7.4. Flagellates grew better and survived longer at pH 7.4. The growth of flagellates was best at 25°C; good growth was also secured at 30°C but at 37°C growth was very poor.

Tr. brucei grew readily on NN, Behrens

dialyzed meat-extract blood agar, Behrens bean-extract blood agar, and NNP medium containing 75, 66 and 50% defibrinated blood. Initial cultures of *Tr. brucei* on NNP-2 tubes were not uniform, although at times positive results were obtained.

The cultural forms of *Tr. brucei* both in the initial culture and subsequent subculture generations were characteristic (See Fig. 2 and 3). The flagellates were noted either as single, free-swimming cells or in stages of division in groups consisting of from two to several hundred cells. Some of the free-swimming forms were very active, others sluggish, with astonishing variations in their size and shapes: short and slender, long and slender, short and broad, and long and broad. The flagellum of the free swimming forms varied; some were very short, others very long. The majority of the flagellates had a healthy protoplasm containing a few to several granules and from four to eight vacuoles. Trypanosomal forms with long, distinct, undulating membranes predominated. Crithidial forms were noted occasionally. Dividing forms of flagellates, and large and small multiple-division forms of rosettes, were noted quite frequently. The rosettes which had very irregular, disorderly shapes resembled medusa heads composed of many flagellates at varying stages of division; they at times reached enormous size and nearly filled the entire microscopic field (45 x 10). As cultures became older, the flagellates became sluggish, highly granular, rounded and eventually died and disintegrated to granules. The viability of cultural forms of *Tr. brucei* in NNP varied from 1 to 3 months.

Tr. brucei grew readily and luxuriantly not only on the water of condensation but also on the slant surface of the subcultures. The majority of these strains were discarded after growing in vitro from 45 to 75 days. During this time they had undergone three to seven subcultivations. A few strains selected as stock cultures have been maintained in vitro for over 3 years and have undergone 25 subcultivations. Examinations of stock cultures revealed that all flagellates maintained their morphologic characteristics throughout all subcultures.

Eleven x-ray-irradiated mice, eight mice injected with aminopterin, 25 normal mice and four normal rats, which were inoculated with cultures of *Tr. brucei* from NN, NNP or Behren's media, representing varying ages and generations of flagellates, failed to contract infection. Frequent microscopic examinations of the tail blood and cultural tests of these animals were invariably negative for *Tr. brucei*.

DISCUSSION

Present studies leave no doubt that *Trypanosoma brucei* can be cultured readily from various experimentally infected animals on NN medium and several of its modifications such as NNN, NNP, etc., provided the parasite is cyclically transmissible by tsetse fly. The two strains used in the present study 4 years after the last passage through tsetse fly still retain their ability to grow in vitro. It appears that if *Tr. brucei* is kept by syringe passage over 25 years in animals without cyclic transmission, the parasite might lose its ability to develop in the tsetse fly and grow in vitro at 20 to 30°C. The fact that the strain of *Tr. brucei* which Novy and MacNeal grew in 1904 and which 10 years afterward was cultured by Behrens could not be grown in vitro later by other investigators can be attributed perhaps to this factor.

It is not certain how long the present strains can be maintained in animals by continuous syringe passage before they will lose the ability to grow in vitro which, in all probability, corresponds to the lack of transmissibility by tsetse fly. It is noteworthy that the present strain of *Tr. brucei* stock is kept in *P. m. gambeli*, and syringe passage is usually made every 3 to 6 months, while Novy made syringe passage every 2 weeks in guinea pigs.

Novy and MacNeal[5] demonstrated that their cultures of *Tr. brucei* were usually infective to laboratory animals. Behrens[2] also reported the the infectivity of *Tr. brucei* cultures to experimental animals. This latter investigator demonstrated that 2 to 3 years of continuous in vitro cultivation of *Tr. brucei* markedly attenuated the parasite, the latter becoming avirulent, except in a few cases, for guinea pigs and rabbits, and less virulent for rats, mice and dogs. The fact that the subcultures obtained in the present studies in vitro failed to produce infection in experimental animals after they were grown on original NN and NNP media, even when rats and mice were exposed to x-ray or aminopterin, suggests that there must be some factor or factors lacking in the ingredients of the present

medium. The peptone or agar used by Novy and MacNeal and by Behrens may have contained some impurities which may, among other factors, contribute to the infectivity of *Tr. brucei* cultures to experimental animals.

It is noteworthy that *Tr. cruzi* grown on NNP and NNP-2 for 13 years without animal passage was still infective to experimental animals[11] and *Tr. lewisi* when grown on NN and NNP media remained infective to rats during the first year of cultivation but at the end of 2 years became completely avirulent and innocuous to rats.[7] On the other hand, *Tr. duttoni* grown on NNP-2 medium for over 2 years is still infective to mice (unpublished data, Packchanian and Smith).

On NNP medium the writer has also grown *Tr. congolense*, *Tr. gambiense* and *Tr. rhodesiense*. The following organisms have also been grown readily both on NNP and NNP-2: *Tr. avium*, *Tr. conorrhini*, *Tr. cruzi*, *Tr. duttoni*, *Tr. melophagium*, *Tr. rangeli*, *Tr. rotatorium*, *Tr. theileri*, *Endotrypanum schaudinni*, *Leishmania donovani*, *L. tropica*, insect flagellates, etc.[7, 10, 14]

SUMMARY

A modified NN medium designated as NNP (Novy, MacNeal and Packchanian) is described for cultivation of trypanosomes in vitro, with special reference to *Trypanosoma brucei*.

Two Uganda strains of *Tr. brucei* were successfully cultured on NN and NNP media directly from 74 experimentally infected animals during 1954 to 1958.

Positive cultures of *Tr. brucei* were obtained on NNP medium two times from guinea pigs, 24 times from rats, 47 times from house mice (*Mus musculus*) and one time from American deer mice (*Peromyscus maniculatus gambeli*).

Stock cultures of *Tr. brucei* have been maintained in vitro on NNP medium by monthly transfer for periods of from 2 months to over 3 years. The flagellates grow readily and luxuriantly not only in the water of condensation but at times on the slant portion of the medium.

The morphology of cultural forms of *Tr. brucei* is characteristic and can at times be differentiated from cultural forms of other species of trypanosomes.

Both initial cultures and subsequent subcultures at varying ages grown on the medium described were not infective to experimental animals.

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ANTIBODIES TO YELLOW FEVER AND OTHER ARTHROPOD-BORNE VIRUSES IN HUMAN RESIDENTS OF SAN VICENTE DE CHUCURI, SANTANDER, COLOMBIA*

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The studies reported here represent the first, or retrospective, phase of a long-term study of the epidemiology of jungle yellow fever (JYF) in San Vicente de Chucurí, Colombia, where the disease has been diagnosed in man 78 times during the 22-year period 1936–1957. The human sera were collected (by HV) between February, 1956, and March, 1957. All sera were tested for yellow-fever (YF) antibody by the intracerebral neutralization test in mice (NT). Most of the sera were also examined by the hemagglutination-inhibition (HI) test of Casals for YF antibody. Neutralization tests and HI tests were done with antigens for ten other arthropod-borne viruses of mammals, mostly of Casals groups A and B.

Description of San Vicente de Chucurí

The town of San Vicente de Chucurí, long. 73°25′20″ W., lat. 6°53′45″N., is the capital of the municipio (county) of the same name with an area of about 679 square km, situated in the western foothills of the Eastern Cordillera of the Andes. The municipio comprises two topographically different areas. The first is the Foothill area, at altitudes ranging from 500 to 1,000 m above sea level, with narrow, steep-sided valleys. Most of this area is drained by the Chucurí River. The second, or Valley Bottom area, lies to the west at altitudes of 150 to 500 meters and includes the markedly dissected old flood plain of the Magdalena River. This area slopes gently toward the Magdalena River, near which there are some extensive swamps (see map).

The climax vegetation of the Valley Bottom area is tropical rain forest, except for the swamps. Along the highway from San Vicente to the river port city of Barrancabermeja the forest has been extensively cleared and the land used for raising cattle.

In the Foothills area the climax vegetation is also tropical rain forest, but very extensive clearing for diversified agricultural purposes has produced a very confused picture, with a wealth of different ecological niches. Coffee and cocoa are the chief cash crops, but they occupy only a small portion of the land. Both of these crops are grown under light shade in Colombia, with the consequent production of special forest conditions. Interspersed between the coffee and cocoa groves are pastures and fields of sugar cane, maize and other food crops. Still present are a few patches of heavy forest, almost virgin. Some of these are continuous with the virgin forest of the Valley Bottom. Another type of forest is the second growth which has appeared on land that is "resting" between periods of shifting cultivation.

The mean temperature of the Foothills is 27°C. The temperature of the Valley Bottom area ranges between 20.8°C and 37.2°C, with a mean of 28.4°C. The mean annual rainfall in the Valley Bottom is about three m (at Barrancabermeja, Santander). With the exception of the data for 1957, there are no official data for the Foothills area, but the rainfall is abundant, because cocoa requires heavy rainfall throughout the year, in addition to high temperature. In the period from November, 1956 to October, 1957, a year considered dry in the region, the total precipitation at San Vicente (town) was 1,428 mm.**

The population of the municipio of San Vicente de Chucurí was 18,375 according to the census of 1951. Most of the population lives in the Foothills area. The Valley Bottom is relatively sparsely populated, but the population has

* The studies and observations here reported were done at the Carlos Finlay Institute, Bogotá, Colombia, a dependency of the Ministry of Public Health of Colombia which also receives financial support and technical advice from the Pan American Sanitary Bureau, and at the Department of Preventive Medicine of the Faculty of Medicine, Universidad del Valle, Cali, Colombia.

† From the Carlos Finlay Institute.
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‖ From the Carlos Finlay Institute.

** Personal communication to Dr. Gast-Galvis, Director, Carlos Finlay Institute from the Chief, Servicio Técnico Agrícola Colombo-Americano in San Vicente de Chucurí.

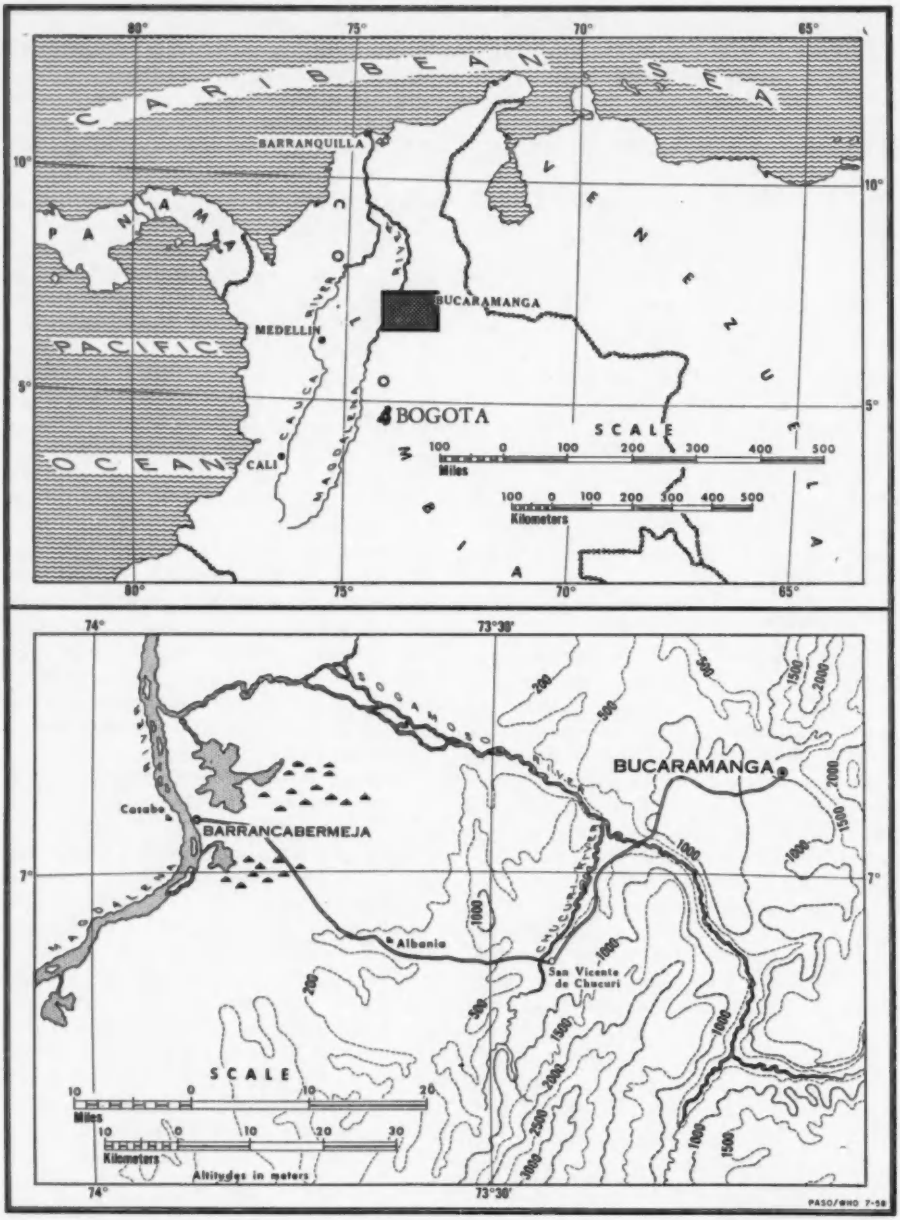

FIG. 1. Outline map of Colombia and detailed map of the San Vicente de Chucurí, Santander, study area

increased rapidly during the past 3 or 4 years as a result of colonization along a new branch highway that has been opened.

In the Foothills area of San Vicente monkeys are either rare or entirely absent. In the places where most of the yellow fever cases have occurred, the local residents say there are no monkeys. However, in some patches of forest connected with jungle of the Valley Bottom a few *Cebus albifrons* and *Alouatta senicula* have been observed. On the contrary, in the Valley Bottom *C. albifrons*, *A. senicula*, *Ateles belzebuth* and *Aotus trivirgatus* are widely distributed but nowhere very abundant. There are no marmosets in either area. Marsupials are frequently observed in both areas, and there is no reason to suspect that the remaining mammalian fauna of the municipio is not the usual one in Colombia.

As to clinical cases of these virus infections, there is evidence that YF and Venezuelan equine encephalitis (VEE) occur in San Vicente. During the 22-year period from 1936 to 1957 a total of 78 fatal cases of YF has been diagnosed by viscerotomy: 56 from the Foothills area; twelve from the Valley Bottom; and ten of unknown origin. These cases have occurred in groups at intervals of 4 to 5 years, with intervening periods of 2 to 3 years without any known cases. This is the usual pattern of JYF in the Magdalena Valley.[7] These outbreaks have occurred in spite of repeated large-scale vaccination against YF in San Vicente.

VEE virus was recovered in 1955 from the blood of a resident of the Valley Bottom who suffered from an acute febrile illness, without any encephalitic symptoms. A second strain of VEE virus was isolated in 1957 under very similar conditions. Both isolations were made at the Carlos Finlay Institute in Bogotá.

MATERIALS AND METHODS

Collection of sera. In the Foothills area 1,189 usable sera were collected from the residents of ten rural areas, or "fracciones," mainly in the valley of the Chucurí River. In the Valley Bottom area 96 sera were collected from people who had lived in the area at least 5 years if they were under 15 years of age, and at least 10 years if they were adults. At San Vicente the sera were kept in an electric refrigerator until they were shipped to Bogotá under refrigeration. At the laboratory in Bogotá the sera were stored at −20°C.

Ninety one specimens of serum were lyophilized and sent to the Rockefeller Foundation Virus Laboratories in New York where they were tested by HI with several group B antigens, including dengue 1 as well as with Mayaro and Chikungunya antigens.

Technique of neutralization tests. The neutralization test technique followed was that described by Smithburn *et al.*,[9] with the exception that the serum-virus mixtures were incubated at 37°C for 1 hour in the case of YF, St. Louis and Ilheus. Furthermore, for the Ilheus virus, the test dilution of the virus was made in undiluted, fresh, normal, guinea pig serum in order to provide the so-called "fresh serum factor." For the dengue virus, all sera were inactivated for 1 hour at 56° C prior to use; for the other viruses the sera were used without inactivation. Groups of six mice were inoculated intracerebrally with 0.03 ml of a mixture containing equal amounts of the serum to be tested and a dilution of virus estimated to contain 100 LD_{50}. In the case of VEE virus 0.1 ml of the serum-virus mixture was inoculated intraperitoneally. No positive results were accepted unless the virus dose used proved to be more than 50 LD_{50} per mouse. Survival ratios of 6/6, 5/6, 5/5, 4/5 and 4/4 were interpreted as positive, all others as negative.

The viruses, and virus strains, used were: yellow fever, French neurotropic (FN) strain; dengue, strain Tr 1751, recently isolated in Trinidad by Anderson, Downs and Hill,[2] which is more closely related to dengue-2 virus than to dengue-1; Ilheus, strain Laemmert; VEE, strain Kubes; St. Louis; EEE; and Bunyamwera. All were supplied by the Rockefeller Foundation Virus Laboratories. Stocks of virus for use in neutralization tests were lyophilized in Colombia and stored at −70°C.

Technique of hemagglutination-inhibition tests. Sera taken at random from the 1,285 specimens were used in HI tests with one or more of the following antigens; yellow fever, pantropic strain JSS; dengue, strain Tr 1751; Ilheus, strain Laemmert; VEE, strain Kubes; WEE; EEE; and Mayaro.

The techniques used were those described by Casals and Brown,[4] as subsequently modified at the Rockefeller Foundation Virus Laboratories.

All sera showing HI in dilutions of 1:10 or higher, using 8 units of an antigen, were considered as positive to that antigen. In order to

eliminate nonspecific inhibitors, the sera were treated with a suspension of bentonite, U.S.P. obtained from Fisher Scientific Company, according to the technique of Clark and Casals.[6] With the exception of the HI tests performed with WEE antigen, all sera which gave positive results after treatment with bentonite were treated with acetone, and then retested. Although no major differences were observed between the results of the test and the retest, occasionally a serum giving an HI titer of 1:10 after treatment with bentonite, was negative at the same dilution after treatment with acetone. In this study, with the aforementioned exception, only the positive results obtained with acetone-treated sera were accepted.

DISCUSSION OF RESULTS

General results. In Table 1 are summarized the results of all the tests performed with the sera from both areas in San Vicente, including the tests with Mayaro, Chikungunya and 17D performed at the Rockefeller Foundation Virus Laboratories. In this table a rough classification according to length of residence has been made. The heading "long residence" includes all individuals of 5 to 14 years of age living in the area for 5 or more years and all individuals 15 years and older living in the area for 10 or more years. The remaining individuals belong to the category "short residence." As stated before, all the individuals from the Valley Bottom area are long residents. This table shows that antibodies for VEE, WEE, Mayaro, YF, Ilheus, Dengue Tr 1751, St. Louis and Bunyamwera were found. No antibodies for EEE and Chikungunya were demonstrated. However, only 27 sera were tested with these two viruses, so the results are not conclusive. High prevalence of NT and HI antibodies for YF in both areas and high prevalence of HI antibodies for VEE, Ilheus and dengue in the Valley Bottom, were demonstrated.

Comparing the results of the sera from the Valley Bottom with those from the Foothills

TABLE 1

Results of tests performed on human sera collected from residents of the Foothills and Valley Bottom areas in San Vicente

Virus used	Test used*	Foothills						Valley Bottom	
		Total		Short residence		Long residence		Long residence	
		Ratio†	% pos	Ratio	% pos	Ratio	% pos	Ratio	% pos
Yellow fever, strain FN	NT	880/1189	73.6	282/438	64.3	598/751	79.6	73/96	76.0
Yellow fever, strain 17D	HI	76/90	84.5	17/22	77.3	59/68	86.8	—	—
Yellow fever, strain JSS	HI	150/609	24.6	40/203	19.7	110/406	27.1	36/93	38.7
Ilheus	NT	25/731	3.4	3/213	1.4	22/518	4.2	14/96	14.6
Ilheus	HI	88/608	14.5	20/199	10.0	68/409	16.6	39/93	42.0
Dengue Tr1751	NT	19/598	3.2	6/169	3.6	13/429	3.0	10/96	10.4
Dengue Tr1751	HI	85/535	15.9	17/164	10.4	68/371	18.3	29/94	30.9
St. Louis	NT	5/85	5.9	1/36	2.8	4/49	8.2	7/86	8.1
Venezuelan Equine Encephalitis	HI‡	18/864	2.1	6/326	1.8	12/538	2.2	32/94	34.1
Venezuelan Equine Encephalitis	HI§	15/864	1.7	1/326	0.3	14/538	2.6	8/94	8.5
Mayaro	HI‡	3/90	3.3	0/21	0	3/69	4.3	—	—
Western Equine Encephalitis	HI‡	0/391	0	0/181	0	0/210	0	—	—
Western Equine Encephalitis	HI§	17/391	4.4	5/181	2.8	12/210	5.7	—	—
Eastern Equine Encephalitis	HI	0/27	0	0/5	0	0/22	0	—	—
Eastern Equine Encephalitis	NT	0/9	0	0/7	0	0/2	0	—	—
Chikungunya	HI	0/27	0	0/5	0	0/22	0	—	—
Bunyamwera	NT	1/201	0.5	1/99	1.0	0/102	0	1/85	1.2

* HI—Hemagglutination-inhibition test; NT = intracerebral neutralization test in mice.

† The numerator indicates number of positive sera, and the denominator the number of sera tested.

‡ In this series only the positives giving titers of 1:80 or more were considered.

§ In this series only the positives giving titers of 1:10 to 1:40 were considered.

(long residence), uncorrected for age and sex, it appears that the prevalence of HI antibodies for VEE, and HI and NT antibodies for Ilheus and dengue is significantly higher in the Valley Bottom.

Casals group B viruses

The results of the NT for YF and three other group B viruses will be discussed first, then the results of HI tests, and finally a discussion will be given of the correlations, or lack of them, that were observed.

Since there is definite evidence of the occurrence of YF virus in San Vicente, the presence of YF neutralizing antibodies is taken to mean that the donor of the serum had been infected with YF virus or had been vaccinated. The validity of this procedure has long been established. The positive results by NT with dengue Tr 1751, Ilheus, and St. Louis are interpreted as probably, but not certainly, indicative of past infection with the respective viruses. Although clinical dengue has been commonly observed in Colombia, and Ilheus and St. Louis viruses have been isolated in several regions of tropical America, no isolations of these viruses have been made in San Vicente, or elsewhere in Colombia. Furthermore, of the sera studied with all four viruses by NT, there were only three single positives: one each for Ilheus, dengue, and St. Louis. The majority of the sera positive for these agents were also positive for YF, which is no surprise, the prevalence of YF positives being so high. The possibility that some of the multiple positives, and even of the single positives, might be due to cross-reactions with known, or still undescribed, group B agents is fully recognized. However it is considered improbable that such possible cross-reactions have seriously affected the interpretation of our results, especially those concerning YF.

Neutralizing antibodies for yellow fever virus. High rates of prevalence of YF antibody were demonstrated by NT both in the Foothills (73.6%) and in the Valley Bottom (76.0%). In the Foothills, as shown in Table 2, the analysis of the results by age, sex and length of residence does not show significant differences with the exception of the people born outside San Vicente who have been living there for less than 1 year. In this group of 115 individuals, only 40 (34.8%) were found to be positive.

The very high prevalence of antibody is the result of two factors: the intensive inoculation of the people with 17D vaccine; and the occurrence of JYF infections, which have been observed frequently in the municipio since 1936. There is no direct evidence to show which of these two factors produced more immunity, but the following information is pertinent.

During the years 1939 to 1956 a total of 55,536 vaccinations with 17D virus were done in San Vicente, 20,888 of them in 1955 alone. Doubtless a great many people were vaccinated more than once because of the repeated vaccination campaigns that were carried out in the municipio.

Precise information regarding prior 17D vaccination of the persons who were bled during the study is not available, even though each person that was bled was carefully questioned about YF vaccination when the blood specimen was taken. Disregarding results on the sera of persons whose 17D vaccination history was "unknown," it was found that of 714 sera from persons who said they were vaccinated, 619 were positive (86.8%); and that of 314 persons who said they had not been vaccinated only 134 were positive (42.7%).

In the "vaccinated" individuals the distribution of positives by age, sex, and length of residence was what would be expected after a mass vaccination of all ages and sexes. In the "unvaccinated" persons both sex and length of residence are of importance, as may be seen from the data summarized in Table 3. In persons 20 years of age and older, and with length of residence of 15 or more years, the proportion of positives is significantly higher in males than in females, and it increases with increasing length of residence. This is the general picture of YF immunity in an unvaccinated community exposed to frequent waves of JYF. Thus it seems probable that relatively few of the "unvaccinated" persons had actually been vaccinated.

In the Valley Bottom the prevalence of NT antibodies for YF is similar to that of the Foothills. In 96 sera there were 73 positives (75.6%), somewhat irregularly scattered throughout the various age and sex groups, as set forth in Table 4. Again the antibody pattern is what would be expected as the result of mass vaccination.

For a picture of the immunity produced by JYF alone, we must turn to the results of a study made in the municipio of Muzo, Colombia in 1936–1937, before any YF vaccination was done in the municipio, and before 17D vaccine was available in Colombia. It was found that 725

TABLE 2

Yellow fever virus. Summary of results of neutralization tests on 1,189 persons resident in the Foothills, by age, sex and length of residence

Length of residence (yrs)		Males Age group (yrs)								Females Age group (yrs)								Grand total
		5–9	10–14	15–19	20–29	30–39	40–49	50+	Total	5–9	10–14	15–19	20–29	30–39	40–49	50+	Total	
Whole life	No. ex.*	61	81	21	19	19	10	9	220	60	74	24	34	20	7	3	222	442
	% pos	77.2	75.4	90.4	94.8	79.0	90.0	77.8	78.7	78.4	70.2	79.2	85.4	70.0	85.8	66.7	76.1	77.4
<1	No. ex.	9	9	17	27	11	2	4	79	6	5	2	15	3	2	3	36	115
	% pos	33.3	55.6	35.3	40.7	45.4	0	0	38.0	0	20.0	50.0	26.6	33.3	50.0	66.7	27.8	34.8
1–4	No. ex.	16	14	21	44	17	12	7	131	6	17	11	32	13	4	6	89	220
	% pos	56.2	85.8	57.2	77.3	82.3	66.7	57.2	71.0	66.7	82.3	54.6	75.0	84.6	50.0	66.7	73.1	71.9
5–9	No. ex.	2	17	14	17	13	10	5	78	2	18	8	20	14	1	1	64	142
	% pos	100.0	58.8	92.9	94.1	92.3	60.0	80.0	80.8	0	83.4	87.5	75.0	71.5	100.0	0	75.0	78.2
10–14	No. ex.	—	4	8	21	21	4	7	65	—	3	5	9	16	6	5	44	109
	% pos	—	100.0	100.0	85.7	85.7	100.0	71.4	87.6	—	100.0	60.0	100.0	93.8	66.7	80.0	86.4	87.1
15–19	No. ex.	—	—	—	7	15	8	3	33	—	—	—	5	9	5	5	24	57
	% pos	—	—	—	100.0	93.4	100.0	100.0	97.1	—	—	—	40.0	55.5	40.0	80.0	54.2	79.0
20–29	No. ex.	—	—	—	3	8	7	3	21	—	—	—	1	16	6	6	29	50
	% pos	—	—	—	100.0	100.0	85.0	100.0	95.3	—	—	—	100.0	87.5	66.7	100.0	86.3	81.9
30+	No. ex.	—	—	—	—	—	7	23	30	—	—	—	—	2	9	13	24	54
	% pos	—	—	—	—	—	100.0	82.7	86.6	—	—	—	—	100.0	88.9	61.5	75.0	81.5
Totals	No. ex.	88	125	81	138	104	60	61	657	74	117	50	116	93	40	42	532	1,189
	% pos	65.9	73.6	71.6	77.6	82.6	80.0	73.8	73.9	68.9	73.6	72.0	72.4	77.4	70.0	71.5	72.6	73.6

* Number examined.

(34.1%) of 2,132 persons residing in Muzo had YF neutralizing antibody in their blood serum (Kerr, Roca-García and Bugher, to be published). Inasmuch as the epidemiology and prevalence of JYF are believed to be quite similar in Muzo and in San Vicente, it is reasonable to conclude that approximately two thirds of the positive sera in San Vicente are due to vaccination. Calculations of the weighted average prevalence of YF antibody in the two areas do not seem warranted, even though census data for the two areas were collected, and are available.

A very important fact emerges from this study: about 25% of the population of San Vicente has no YF antibody demonstrable in its blood serum (based on a very conservative interpretation of NT results). It is assumed that all persons with demonstrable amounts of YF neutralizing antibody in their blood serum are immune to YF. This finding means that the vaccination program fails to deliver vaccine to an appreciable portion of the population that needs it. There is no reason to think that once 17D virus is inoculated into a person it fails to immunize him against YF. The fact is that YF virus continues to find people before the YF vaccinators do, with unfortunate results in a disturbingly large number of instances. Without attempting to pass judgment, or discuss the many factors which enter into the present situation, the conclusion must be drawn that the ideal vaccination procedures have not yet been put into practice.

TABLE 3

Yellow fever virus. Results of neutralization tests on sera from 192 individuals resident in the Foothills, aged 20 years and over, who stated they had not been vaccinated against yellow fever, analyzed by sex and length of residence

Length of residence (yrs)	Males		Females	
	No. pos./ No. exam.	% pos.	No. pos./ No. exam.	% pos.
1	7/35	20.0	5/20	25.0
1–4	10/19	52.6	9/19	47.3
5–14	10/18	55.6	11/17	64.7
15 and up	23/30	76.7	18/34	52.9
Totals.....	50/102	49.5	43/90	47.8

Neutralizing antibodies for dengue Tr 1751 virus. The prevalence of NT antibodies to dengue virus is summarized by age, sex, and length of residence in Table 5. In the Foothills area there were 19 positive sera among 598 tested (3.2%). The departures from this mean are not statistically significant, but it is of interest to note that of 254 persons, of both sexes, aged 5–29 years, who had lived their whole lives in the Foothills, only one had dengue neutralizing antibodies. This finding indicates that during the past 30 years the chance of acquiring dengue antibodies in the Foothills has been practically nil.

In the Valley Bottom 10% of 30 males aged 5–29 years who have lived all their lives in the area have dengue antibodies in their sera. Of

TABLE 4

Yellow fever virus. Summary of results of neutralization tests on 96 persons resident in the Valley Bottom, by age, sex and length of residence

Length of residence		Males Age group (yrs)						Females Age group (yrs)						Grand totals
		5–9	10–14	15–19	20–29	30+	Total	5–9	10–14	15–19	20–29	30+	Total	
Whole life	Ratio*	4/5	16/19	2/2	2/2	—	24/28	8/9	6/6	1/2	—	—	15/17	39/45
	% pos	80.0	84.3	100.0	100.0	—	85.7	88.9	100.0	50.0	—	—	88.4	86.7
5+ years	Ratio	1/2	7/10	1/3	0/1	10/15	19/31	—	2/2	—	1/2	12/16	15/20	34/51
	% pos	50.0	70.0	33.3	0	66.7	61.3	—	100.0	—	50.0	75.0	75.0	66.7
Totals	Ratio	5/7	23/29	3/5	2/3	10/15	43/59	8/9	8/8	1/2	1/2	12/16	30/37	73/96
	% pos	71.4	79.3	60.0	66.7	66.7	73.9	88.9	100.0	50.0	50.0	75.0	81.1	76.0

* The numerator indicates the number of sera giving positive results; the denominator, the total number of sera tested.

TABLE 5

Dengue virus, strain Tr1751. Results of neutralization tests on 694 sera, analyzed by age, sex and length of residence. Foothills and Valley Bottom

Length of residence		Males Age group (yrs)					Females Age group (yrs)					Grand totals
		5–9	10–14	15–29	30+	Total	5–9	10–14	15–29	30+	Total	
Foothills												
Whole life	Ratio*	1/39	0/62	0/25	1/24	2/150	0/41	0/51	0/36	4/22	4/150	6/300
	% pos.	2.6	0	0	4.2	1.3	0	0	0	18.2	2.7	2.0
0–4 years	Ratio	0/9	0/13	0/39	2/19	2/80	0/6	0/10	1/18	1/12	2/46	4/126
	% pos.	0	0	0	10.5	2.5	0	0	5.6	8.3	4.3	3.2
5+ years	Ratio	0/2	0/5	0/24	5/54	5/85	0/1	0/18	0/22	4/46	4/87	9/172
	% pos.	0	0	0	9.3	5.9	0	0	0	8.7	4.6	5.2
Totals	Ratio	1/50	0/80	0/88	8/97	9/315	0/48	0/79	1/76	9/80	10/283	19/598
	% pos.	2.0	0	0	8.3	2.9	0	0	1.3	11.2	3.5	3.2
Valley Bottom												
Whole life	Ratio	1/6	1/20	1/4	—	3/30	0/9	0/6	0/2	—	0/17	3/47
	% pos.	16.7	5.0	25.0	—	10.0	0	0	0	—	0	6.4
5+ years	Ratio	0/2	0/9	0/4	5/15	5/30	—	0/2	0/2	2/15	2/19	7/49
	% pos.	0	0	0	33.3	16.7	—	0	0	13.3	10.5	14.3
Totals	Ratio	1/8	1/29	1/8	5/15	8/60	0/9	0/8	0/4	2/15	2/36	10/96
	% pos.	12.5	3.5	12.5	33.3	13.3	0	0	0	13.3	5.6	10.4

* The numerator indicates the number of sera giving positive results; the denominator, the total number of sera tested.

the total of 96 sera tested ten (10.4%) were positive for dengue. This prevalence is significantly greater than in the Foothills. The positive results are scattered among the groups of houses in a highly random fashion, giving no suggestion of "house epidemics."

The Valley Bottom area is in easy communication with Barrancabermeja and other Magdalena River bank towns, in which it must be assumed that dengue fever was prevalent until just a very few years ago, when *Aedes aegypti* was eradicated from the area.

Therefore, in spite of the rural residence of the donors in the Valley Bottom, and even in the Foothills, and assuming that the antibodies were actually due to a past dengue infection, the possibility must be considered that the dengue infections were acquired in towns from *Aedes aegypti* mosquitoes, and not in the forest. It is concluded that the available data do not constitute proof of the presence of "jungle dengue" in San Vicente de Chucurí.

Neutralizing antibodies for Ilheus virus. The prevalence of Ilheus antibody in 731 sera from the Foothills and in 96 from the Valley Bottom is summarized in Table 6. In the Foothills there were 25 positives (3.4%) and in the Valley Bottom 14 (14.6%). Among 236 children 5–14 years of age who had lived their whole lives in the Foothills, four (1.7%) were positive for Ilheus. Assuming that the antibodies were due to a past infection with Ilheus virus, the deduction seems warranted that these children acquired their infection in the Foothills. The concept that Ilheus virus is present in the Foothills is supported by the observation that the prevalence of Ilheus antibodies increases somewhat with increasing length of residence and age.

In the Valley Bottom the crude prevalence rate is significantly higher than in the Foothills, but the partial rates shown in Table 6 are not meaningful. However, in the several groups of houses mentioned later in the discussion of VEE, there are some interesting variations, to wit: the ratios of immunes in the dwellers of the five groups of houses along the road were: 6/11

TABLE 6

Ilheus virus. Results of neutralization tests on 827 sera, analyzed by age, sex and length of residence. Foothills and Valley Bottom

Length of residence		Males Age groups (yrs)					Females Age group (yrs)					Grand Total
		5–9	10–14	15–29	30+	Total	5–9	10–14	15–29	30+	Total	
Foothills												
Whole life	Ratio*	1/50	1/73	3/29	1/27	6/179	1/49	1/64	0/42	2/21	4/176	10/355
	% pos.	2.0	1.4	10.3	3.8	3.4	2.0	1.6	0	9.5	2.3	2.8
0–4 years	Ratio	0/12	0/16	0/47	1/20	1/95	0/7	0/11	0/29	0/16	0/63	1/158
	% pos.	0	0	0	5.0	1.1	0	0	0	0	0	0.6
5+ years	Ratio	0/2	0/9	3/38	7/63	10/112	0/1	0/17	0/27	4/61	4/106	14/218
	% pos.	0	0	7.9	11.1	8.9	0	0	0	6.6	3.8	6.4
Total	Ratio	1/64	1/98	6/114	9/110	17/386	1/57	1/92	0/98	6/98	8/345	25/731
	% pos.	1.6	1.0	5.3	8.2	4.4	1.8	1.1	0	6.1	2.3	3.4
Valley Bottom												
Whole life	Ratio	1/6	1/20	3/4	—	5/30	2/8	0/6	0/2	—	2/16	7/46
	% pos.	16.7	5.0	75.0	—	16.7	25.0	0	0	—	12.5	15.2
0–4 years	Ratio	0/2	1/9	0/4	3/15	4/30	—	0/2	0/2	3/16	3/20	7/50
	% pos.	0	11.1	0	20.0	13.3	—	0	0	18.8	15.0	14.0
Total	Ratio	1/8	2/29	3/8	3/15	9/60	2/8	0/8	0/4	3/16	5/36	14/96
	% pos.	12.5	6.9	37.6	20.0	15.0	25.0	0	0	18.8	13.9	14.6

* The numerator indicates the number of sera giving positive results; the denominator, the total number of sera tested.

(54.5%); 1/19 (5.3%); 4/15 (27%); 0/6; and 0/12. The figure for the dwellers of the houses in the forest was 2/16 (12.5%).

Ilheus virus has been isolated several times from *Psorophora ferox*.[1, 8] This mosquito is more abundant in the Valley Bottom than in the Foothills. The higher prevalence and irregular distribution of antibodies may, perhaps, be correlated with the abundance of this mosquito.

Neutralizing antibody for St. Louis virus. As shown in Table 1, of 85 sera selected at random from Foothills residents, NT antibodies for St. Louis were demonstrated in 5 (5.9%) and of 87 from the Valley Bottom, 7 (8.1%) were positive. A careful analysis of this small sample by age, sex and length of residence reveals nothing of special interest. It seems probable that St. Louis virus has been active both in the Foothills and in the Valley Bottom.

HI antibodies for group B viruses, in general. Casals and Brown[4] called attention to the considerable degree of cross reactions that occur with group B antigens in the HI test. This fact makes the HI test very useful for showing similarities between viruses in contrast with the NT, which is specially useful for showing differences between viruses.

More recently Casals[3] has pointed out that the immune response to an infection with a second group B virus is much more intense and much broader than the response after a first infection.

In San Vicente there is presumptive proof of presence of four of the viruses of group B: YF, dengue, Ilheus, and St. Louis. And it is possible that 17D may act as a fifth virus, especially if the infection with it (*i.e.*, vaccination) precedes an inoculation of feral YF virus by a mosquito.

HI antibody for Yellow Fever virus. In Bogotá the YF antigens used were prepared with the pantropic JSS strain of virus. Of 609 Foothill sera tested only 150 (24.7%) gave positive results. Of 93 Valley Bottom sera only 36 (38.7%) gave positive results. Both these percentages are very much lower than the percentages posi-

tive by NT, which were 73.6% and 76.0%, respectively (see Table 1).

The results on the sample of specimens sent to New York and examined there with a 17D antigen were very different: 76 of 90 specimens (84.5%) were positive.

As shown in Table 7, there is excellent correlation between the HI results with 17D antigen and with NT results with French neurotropic virus. The YF antibody that is present can be the result either of a natural infection with JYF or of a 17D vaccination, in unknown proportions.

The much smaller proportions of HI positives (25%) with JSS antigen is surprising. We are informed by Dr. Max Theiler that, for reasons that have not been determined, the HI antigen prepared from JSS virus is relatively "insensitive" in comparison with antigens prepared from a number of other strains of pantropic YF virus.

A point of importance is that with both the 17D HI tests and the FN neutralization tests, the proportion of positives is about the same in all age groups. This is the picture that would be produced by extensive YF vaccination in a community.

With the JSS hemagglutination-inhibition antigen the proportion of positives is significantly lower in the age group 5–9 years than in that over 30 years. This is the general picture that would be expected in the case of natural infections. It must be admitted that the picture for the intermediate years is confused.

The results of all the HI tests done on Foothill sera with JSS antigen are set forth in Table 8. These 609 sera include the 90 sera summarized in Table 7. Only 150 (24.6%) of these 609 sera are positive, a very different figure from the 73.6% positive by NT, shown in Table 2. Equally striking is the fact that the percentage of HI positives increases with age and length of residence. This is in marked contrast to the NT results, which are approximately equal in all age groups. The proportion of HI positives is about the same in both sexes.

Whatever the real explanations, we are im-

TABLE 7

Results of tests performed on 90 sera from two "fracciones", San Vicente Foothills. Donors: 45 males and 45 females; 43 resident whole life

Virus and test used		Age group (*yrs*)					Totals
		5–9	10–14	15–19	20–29	30+	
All tests	No. tested	14	26	9	10	31	90
NT, yellow fever, strain FN	No. pos.	10	23	6	5	25	69
	No. inc.	3	1	2	1	0	7
	% pos.	71	89	67	50	81	78
	% pos. and inc.	92	91	78	60	81	84
HI, yellow fever, strain 17D*	No. pos.	11	24	8	7	26	76
	% pos.	79	91	89	70	84	84
HI, yellow fever, strain JSS	No. pos.	2	4	2	1	13†	22
	% pos.	14	15	22	10	43	25
NT, Ilheus, strain Laemmert	No. pos.	1	1	0	1	7	10
	% pos.	7	4	0	10	23	11
HI Ilheus, strain Laemmert	No. pos.	1	1	1	2	13	18
	% pos.	7	4	11	20	42	20
HI, dengue, strain Tr 1751	No. pos.	1	1	1	3	3	19
	% pos.	7	4	11	30	42	21

* Tests performed at the Rockefeller Foundation Virus Laboratory, New York.
† Only 30 sera tested.

TABLE 8

Yellow fever virus. Summary of results of HI tests with JSS antigen on 609 persons resident in the Foothills, by age, sex and length of residence

Length of residence (yrs)		Males						Females						Grand totals
		Age group (yrs)						Age group (yrs)						
		5–9	10–14	15–19	20–29	30+	Totals	5–9	10–14	15–19	20–29	30+	Totals	
Whole life	Ratio	3/32	5/50	4/14	1/9	12/21	25/126	4/31	6/37	2/12	3/17	9/20	24/117	49/243
	% pos.	9.4	10.0	28.6	11.1	57.2	19.8	12.9	16.2	16.7	17.6	45.0	20.5	20.2
1	Ratio	1/5	1/5	1/9	2/11	1/8	6/38	0/1	1/3	0/1	1/5	1/3	3/13	9/51
	% pos.	20.0	20.0	11.1	18.2	12.5	15.8	0	33.3	0	20.0	33.3	23.1	17.6
1–4	Ratio	0/3	1/6	3/9	1/11	8/20	13/49	1/5	2/13	2/4	3/15	1/10	9/47	22/96
	% pos.	0	16.7	33.3	9.1	40.0	26.6	20.0	15.4	50.0	20.0	10.0	19.1	22.9
5–9	Ratio	0/1	0/8	0/8	1/11	7/12	8/40	—	0/9	0/4	1/11	0/10	1/34	9/74
	% pos.	0	0	0	9.1	58.3	20.0	—	0	0	9.1	0	2.9	12.2
10–14	Ratio	—	1/3	2/4	4/11	7/15	14/33	—	0/5	—	1/3	3/9	4/17	18/50
	% pos.	—	33.3	50.0	9.8	46.7	42.4	—	0	—	33.3	33.3	23.5	36.0
15–19	Ratio	—	—	—	3/4	4/18	7/22	—	—	—	2/3	4/9	6/12	13/34
	% pos.	—	—	—	75.0	22.2	31.8	—	—	—	66.7	44.4	50.0	38.2
20–29	Ratio	—	—	—	—	5/8	5/8	—	—	—	0/1	8/17	8/18	13/26
	% pos.	—	—	—	—	62.5	62.5	—	—	—	0	47.0	44.4	50.0
30+	Ratio	—	—	—	—	11/21	11/21	—	—	—	—	6/14	6/14	17/35
	% pos.	—	—	—	—	52.3	52.3	—	—	—	—	42.8	42.8	48.6
Totals	Ratio	4/11	8/72	10/44	12/57	55/123	89/337	5/37	9/67	4/21	11/55	32/92	61/272	150/609
	% pos.	9.8	11.1	22.7	21.1	44.7	26.4	13.5	13.4	19.0	20.0	38.4	22.4	24.7

pressed by the fact that, among the 101 males whose sera were submitted to HI tests and to NT with YF, Ilheus, and dengue, and who showed no NT antibodies for Ilheus and dengue, again a higher proportion of hemagglutination-inhibition JSS positives was observed in the older group, as is shown in Table 9. It must be admitted that the picture for females in the same category is confused. With regard to these males it would appear that one can rule out the possibility that the JSS hemagglutination-inhibition results were the consequence of heterologous reactions with Ilheus or dengue, Tr 1751.

HI antibody for Ilheus and Dengue Tr 1751. In the Foothills, of 608 sera 88 (14.5%) gave positive results in HI tests with Ilheus antigen and of 535 specimens 85 (15.9%) gave positive results in HI tests with dengue antigen. The distribution of the positives with both antigens follows a pattern similar to that of the JSS hemagglutination inhibition antibodies, *i.e.*, the proportion of positives increases significantly with age and with length of residence. Since there is a poor correlation between JSS hemagglutination-inhibition results and Ilheus HI and dengue HI results we conclude that the HI antibodies for Ilheus and dengue are not solely the result of YF and 17D virus infections.

In the Valley Bottom the prevalence of HI antibodies for Ilheus (42% of 93 individuals) and for dengue (30.9% of 94 persons) are significantly greater that in the Foothills. Here, again, there is poor correlation between the HI results with the three antigens although it must be noticed that no single HI positives for dengue were observed, which would mean that in this area the dengue HI results are more influenced by heterologous agents than in the Foothills (see Table 10).

As for any correlations between HI and NT with Ilheus as well as with dengue, it is observed that all, or nearly all, the sera positive by NT were positive also by HI with the corresponding antigen, but there were many positive by HI and negative by NT.

To summarize, the HI results indicate clearly that in the Valley Bottom there has been a greater activity of group B viruses than in the Foothills. From the HI results only, it is impossible to determine with precision which viruses

TABLE 9

Results of serological tests on 187 residents of San Vicente Foothills (all with more than 5 years of residence) who showed no neutralizing antibodies for Ilheus or for dengue Tr 1751

Virus and test used		Males						Females						Grand totals
		Age group (yrs)						Age group (yrs)						
		5-9	10-14	15-19	20-29	30+	Total	5-9	10-14	15-19	20-29	30+	Total	
All tests	No. tested	14	33	8	13	33	101	14	29	6	12	25	86	187
NT, YF	No. pos.	9	24	6	11	29	79	13	20	5	8	22	68	147
	% pos.	64.3	72.8	75.0	84.6	87.9	78.2	92.9	69.0	83.3	66.7	88.0	79.1	78.6
HI, YF,	No. pos.	1	2	1	3	12	19	4	2	1	1	6	14	33
strain JSS	% pos.	7.1	6.1	12.5	23.1	36.4	18.8	28.6	6.9	16.7	8.3	24.0	16.3	17.6
HI, Ilheus	No. pos.*	1†	0	0	1†	4‡	6	0	0	0	0	3†	3	9
	% pos.	7.1	0	0	7.7	12.5	5.9	0	0	0	0	12.0	3.5	4.8
HI, dengue,	No. pos.	1	0	0	1	4	6	0	0	0	0	3	3	9
Tr 1751	% pos.	7.1	0	0	7.7	12.5	5.9	0	0	0	0	12.0	3.5	4.8

* All HI positive for Ilheus were also HI positive for dengue and for JSS.
† These sera were also tested with St. Louis virus by NT; in all cases the results were negative.
‡ Two of these sera were tested with St. Louis by NT; the results were negative.

TABLE 10

Results of HI tests with yellow fever (strain JSS), Ilheus, and dengue Tr 1751 antigens on 527 sera from residents of San Vicente

	Foothills		Valley Bottom	
	No.	%	No.	%
Negative to all three antigens	360	68.3	47	50.5
Positive to all three antigens	61	11.6	24	25.8
Positive to JSS and Ilheus only	7	1.3	5	5.4
Positive to JSS and Dengue only	5	0.9	1	1.1
Positive to Dengue and Ilheus only	6	1.1	4	4.3
Positive to JSS only	69	13.1	6	6.5
Positive to Ilheus only	7	1.3	6	6.5
Positive to Dengue only	12	2.3	0	0
Totals	527	99.9	93	100.1

are involved, since they represent principally the combined activity of the several group B agents present. However, these results are suggestive that the greater activity of group B viruses in the Valley Bottom is due mainly to YF and Ilheus viruses. The latter possibility was confirmed by the results of NT with Ilheus.

Casals Group A Viruses

VEE virus. In Table 11 the results of HI tests with VEE virus are analyzed by age, sex and length of residence in the two areas. It will be seen immediately that the prevalence of VEE antibody is very much higher in Valley Bottom residents than in Foothill residents. There is no significant difference between the sexes, and there is a general tendency for the proportion of positives to increase with age.

In the Foothills, of 313 individuals living their whole lives in the area, 263 were 5–29 years of age and 50 older than 29 years. In the first group only one positive was found (0.4%) while in the second there were 4 positives (8.0%). This difference is statistically significant. These findings suggest there have been very few opportunities for the residents of the Foothills to acquire VEE infection. The one positive case in the group aged 5–29 years could be explained as the result of exposure outside the foothills. Even though these people stated they had lived their whole lives in the Foothills, they may have visited the neighboring areas where we know the virus exists. The higher prevalence of antibody in older individuals could be explained as the result of activity of the virus in the Foothills 30 or more years ago, provided the HI antibodies persist for such a long time, which we do not know, or to greater exposure to the infection outside the Foothills. In fact older people usually

TABLE 11

VEE virus. Results of HI tests on 958 human sera, analyzed by age, sex and length of residence. Foothills and Valley Bottom

Length of residence		Males Age group (yrs)					Females Age group (yrs)					Grand totals
		5–9	10–14	15–29	30+	Totals	5–9	10–14	15–29	30+	Totals	
Foothills												
Whole life	Ratio*	0/42	0/61	0/30	3/26	3/159	0/44	1/42	0/44	1/24	2/154	5/313
	% pos.	0	0	0	11.5	1.9	0	2.4	0	4.2	1.3	1.6
0–4 years	Ratio	0/14	0/19	0/83	1/43	1/159	0/7	0/17	3/41	0/22	3/87	4/246
	% pos.	0	0	0	2.3	0.6	0	0	7.3	0	3.4	1.6
5+ years	Ratio	0/2	0/14	1/61	5/108	6/185	0/1	0/15	0/33	3/71	3/120	9/305
	% pos.	0	0	1.6	4.6	3.2	0	0	0	4.2	2.5	3.0
Totals	Ratio	0/58	0/94	1/174	9/177	10/503	0/52	1/74	3/118	4/117	8/361	18/864
	% pos.	0	0	0.6	5.1	2.0	0	1.4	2.5	3.4	2.2	2.1
Valley Bottom												
Whole life	Ratio	2/6	5/19	0/4	—	7/29	1/9	3/6	1/2	—	5/17	12/46
	% pos.	33.3	26.3	0	—	24.2	11.1	50.0	50.0	—	29.4	26.1
5+ years	Ratio	0/2	3/8	2/4	7/14	12/28	—	1/2	1/2	6/16	8/20	20/48
	% pos.	0	37.5	50.0	50.0	42.8	—	50.0	50.0	37.5	40.0	41.7
Totals	Ratio	2/8	8/27	2/8	7/14	19/57	1/9	4/8	2/4	6/16	13/37	32/94
	% pos.	25.0	29.6	25.0	50.0	33.4	11.1	50.0	50.0	37.5	35.1	34.1

* Ratio. The numerator indicates the number of sera giving positive results with titers 1:80 or more when tested with 8 units of antigen. The denominator indicates the total number of sera tested.

have more chances to travel and to visit neighboring areas than younger people.

Now it should be noted that the positives in Table 11 are only those sera that gave an HI titer of 1:80 or higher with 8 units of antigen. It is stated by Casals[3] that the degree of crossed HI reactions between viruses of group A is slight, and much less than between group B viruses. But there is enough cross-reaction between the group A viruses to require analysis.

In sera from the Foothills 18 were HI positive for VEE in titers of 1:80 or more, and 15 in titers of 1:10 to 1:40, all obtained in tests with 8 units of antigen. Of the 18 positives, 16 had HI titers of 1:320 or more. It was not possible to test all these sera by NT, but a few tests were done. Four of the 16 high-titer sera were tested; all gave positive NT results with VEE virus. It is of interest that 8 of these 18 sera were HI tested with WEE antigen, with the following results: 4 negative and 4 positive, with titers of 1:10 and 1:20.

Of the 15 sera giving titers of 1:10 to 1:40 with VEE antigen five were NT tested with VEE virus; one was positive, four were negative. These four negative sera were HI tested with Mayaro and WEE antigens. All were strongly positive for Mayaro with titers of 1:320 or more, and all were weakly positive for WEE with titers of 1:20 and 1:40.

Of 831 sera negative by HI for VEE 84 were tested against VEE virus by NT, with entirely negative results.

Of the 94 Valley Bottom sera 32 were HI positive in high titer for VEE. Eighteen of the positives were submitted to NT with VEE with 18 positive results. Six of the eight low-titer sera were examined by NT, with the following results: four positive, one inconclusive and one negative. The sera from the Valley Bottom were not routinely HI tested with any group A antigen other than VEE.

We conclude that an HI titer of 1:80 or higher, obtained with 8 units of VEE antigen, is the result of an infection by VEE virus; and that HI titers of 1:10 to 1:40 with VEE antigen

indicate merely an infection by some group A virus.

The people bled in the Valley Bottom came from five groups of houses situated in Albania, near the highway from Barrancabermeja to San Vicente, in places where the forest has been cleared, and from one group of houses about 5 miles from the highway, surrounded by forest. The ratios of VEE immunes in the five groups of houses along the road were 1:10 (10%), 5:18 (28%), 4:15 (27%), 1:5 (20%) and 4:12 (33%). The figure for the houses in the forest was significantly higher: 12:17 (71%). The distribution of the population in the various groups of houses was essentially the same so far as sex, age and length of residence are concerned. In this analysis no consideration was given to 17 children bled in one school-house because of lack of exact information about the location of their homes.

It seems evident that in San Vicente VEE infection is a disease associated with rain forest. This is corroborated by the fact that HI antibodies for VEE were found in 11 of 17 sera from monkeys shot in the forests of the Valley Bottom. The titers of these sera were: more than 1:160 in three; 1:80 in two; and 1:10 to 1:40 in six.

Mayaro virus. All the information about this virus was obtained by HI. Three of 90 Foothill sera tested were positive, all in high titer; one of 1:80 and two of 1:640 or more. With VEE by HI two of these sera were negative and the third positive at 1:10; by NT all were negative.

It is concluded that the three persons whose sera were HI positive for Mayaro had been probably infected by that virus, certainly in Colombia but not necessarily in the Foothill area where they resided.

WEE virus. In Table 1 it is shown that 17 of 391 Foothill sera had HI antibodies for WEE virus, all in low titer of 1:10 to 1:40. A study of the cross-reactions of 13 of these 17 sera with VEE and Mayaro antigens reveals that seven were highly positive with Mayaro and five highly, and one slightly, positive for VEE. The remaining four sera were either negative or nottes ted. It is concluded that all the WEE antibody found by HI was due to heterologous viruses, and that there is no evidence of the presence of WEE virus in San Vicente.

Ungrouped viruses

Bunyamwera. Of 201 sera from the Foothills and of 99 sera from the Valley Bottom, two (one from each area) proved to have NT antibodies for Bunyamwera virus. The significance of this finding is not known; however one might speculate that a Bunyamwera-like virus occurs in the area. Recently Causey and Theiler[5] showed that in a group of 232 sera collected from the Amazon Valley 11 contained NT antibodies for this virus.

SUMMARY

The Municipio of San Vicente de Chucurí, Santander, Colombia, is situated partly in the Foothills of the Andes and partly in the Valley Bottom of the Magdalena River. The climate is tropical and the original climax vegetation was tropical rain forest. Since 1936, 78 fatal cases of jungle yellow fever (JYF) have been diagnosed in the municipio by viscerotomy.

This serological study of 1,189 human sera from residents of the Foothills and of 96 sera from residents of the Valley Bottom is the first phase in a long-term study of the epidemiology of JYF in the municipio, which is typical of the JYF area in the Magdalena Valley.

Of the 1,189 sera tested from Foothill residents by yellow fever (YF) neutralization tests (NT) 73.6% were positive, and of the 96 sera from Valley Bottom residents 76.0% were positive. All age groups were about equally positive, as would be expected as the result of the repeated and intensive YF vaccination which has geen done in the municipio.

However, about a fourth of the population is still without demonstrable protection against YF, as judged by very strictly interpreted NT results.

Thus, it is clear that the ideal procedures for applying 17D vaccine have not yet been put into practice in San Vicente.

Quite different results were obtained by the hemagglutination-inhibition (HI) test for YF using an antigen prepared from the pantropic JSS strain of virus. Of 609 representative Foothill sera tested only 24.6% were positive, and of 93 Valley Bottom sera only 38.7% were positive. Not only was the proportion of positives markedly lower in the HI test, but the distribution of the positives was significantly different. The proportion of positives increased with age and increasing length of residence. The HI results on the sample of 90 sera tested with a 17D HI antigen parallel very closely the NT results on the same sera. The significance of these differences has not been ascertained.

Since there is a poor correlation between JSS HI results and Ilheus HI and dengue HI results, we conclude that the HI antibodies for Ilheus and dengue are not exclusively the result of YF and 17D virus infections. The positive NT results with dengue Tr 1751, Ilheus and St. Louis are interpreted as *not* necessarily indicative of past infection with those viruses because only three of the sera studied with all four viruses by NT were single positives: one each of Ilheus, dengue Tr 1751 and St. Louis. It would appear that unless and until dengue, Ilheus and St. Louis viruses, or possibly some other group B virus, are isolated in San Vicente, this doubt cannot be cleared up.

With VEE virus, HI tests were positive in 2.1% of 864 Foothill sera and in 34.1% of 94 Valley Bottom sera. This difference is striking and is interpreted to mean that VEE virus probably does not invade the Foothills area. An HI titer of 1:80 or higher is taken as diagnostic of VEE, and of other group A viruses; lower titers are interpreted as heterologous reactions with other group A viruses.

With Mayaro virus three of 90 Foothill sera were positive by HI. No Valley Bottom sera were tested with this virus. No evidence of activity of either EEE or WEE virus was obtained.

Chikungunya is a group A virus from Tanganyika which has been found to be broadly antigenic in other geographical areas. The number of sera tested is too small to give significance to the negative results.

The significance of the two positive results by HI with Bunyamwera virus is not known. The virus is "ungrouped" by Casals and is of Central African origin. Low proportions of positives are frequently found in groups of sera from South America.

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ADDENDUM

Recent studies by Casals, still unpublished, show that Bunwamwera virus is antigenically related to Wyeomyia virus, known only from Colombia.

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FURTHER STUDIES ON THE EXPERIMENTAL TRANSMISSION OF YELLOW FEVER BY *SABETHES CHLOROPTERUS**

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In 1956 Galindo, Rodaniche and Trapido[3] reported the first experimental transmission of yellow fever by *Sabethes chlorpterus*. Virus was transmitted to one *Macaca mulatta* by the bites of 11 of these mosquitoes after an extrinsic incubation period of 27 to 34 days. A second effort to transmit to a rhesus monkey failed. Failure also was experienced in all attempts to recover virus by intracerebral injection of white Swiss mice, principally young adults, with suspensions of single mosquitoes or small pools. The need was felt for further investigation to clarify the potentialities of *S. chloropterus* as a vector of yellow fever. Since this initial report three additional experiments have been performed which have resolved many of the questions raised in earlier work. In general the same techniques were followed as were formerly employed.

MATERIALS AND METHODS

Virus strains. In the first of the three experiments here reported the Trinidad #4202 strain previously described was again employed. This virus was received in 1954 through the courtesy of Dr. Wilbur Downs, Director of the Trinidad Regional Virus Laboratory, as a suspension of the spleen of a howler monkey found dead in the forest. In the remaining two experiments, a yellow fever strain, C-10, isolated locally, was substituted. This virus was recovered from *Sabethes chloropterus* found naturally infected in Cerro Azul in September, 1956.

Mosquitoes. The *Sabethes chloropterus* were later generations of the same colonized strain used in the previous study. The wild stock for initiation of the colony was captured at Cerro La Victoria in Panama in 1955 and was maintained in a closed insectary, the temperature of which varied between 23°C and 30°C and the relative humidity between 70 and 100%. Infected mosquitoes were kept in large cages in a separate room, completely isolated from the breeding stock and the outside environment by double screen doors.

Serologically negative, healthy, young, rhesus monkeys were employed as the source of infective blood for the initial mosquito feedings. These monkeys were injected subcutaneously with yellow fever virus 2 to 4 days prior to exposure to *S. chloropterus*. The virus content of the blood was titrated in adult mice daily. The monkeys were immobilized on a special board which was placed inside the mosquito cage. Miltown was administered in the first two experiments to tranquilize the animals but its use was discontinued due to fear that it might be having a deleterious effect on the mosquitoes, which showed an unusually high mortality. Great difficulty was experienced in early experimental work in inducing *Sabethes* to feed on monkeys. It was subsequently found[1] that the peak of biting activity does not occur until the second or third week, or even as much as 30 days, after emergence. The initial difficulty was overcome by using older females.

After suitable extrinsic incubation periods, the *Sabethes* were refed on normal monkeys. Young *Macaca mulatta* were used for this purpose in the first two experiments. In the third, juvenile black spider monkeys, *Ateles geoffroyi grisescens* Gray, captured in the wild stage and giving negative protection tests, were substituted.

Mosquitoes found dying or recently dead after an opportunity to become infected were ground with rhesus-serum-saline solution, either singly or in small pools, and injected intracerebrally into white Swiss mice. The quantity of diluent was always 0.6 ml regardless of whether single or pooled insects were used, as this was considered a minimal quantity for our purpose.

Mice becoming ill after an incubation period of more than 4 days were sacrificed. Smears stained with Giemsa, and bacteriological cultures of cerebral tissue, were made and the brain was then emulsified for injection into a group of five normal mice and five mice previously im-

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munized against Theiler's French neuroadapted strain of yellow fever virus. Identification of virus was considered adequate when smears showed no microorganisms, cultures were negative for bacteria, and the immune animals were still alive and well after the nonimmunes had sickened or died. Mice were kept under observations for a period of 28 days. At the end of this time survivors were reinjected intracerebrally with 10,000 LD/50 of French neuroadapted virus to discover possible inapparent infections.

RESULTS

Experiment I

In the first of the transmission experiments here reported (see Table 1) *S. chloropterus* were allowed to feed on a rhesus monkey on the 2nd, 3rd and 4th days after inoculation with the Trinidad #4202 strain in its second monkey passage, when the blood titers of virus were $10^{-5.2}$, $10^{-5.8}$ and $10^{-4.3}$, respectively. The mosquitoes fed very poorly, as this was previous to the use of older females. After an extrinsic incubation period of 32 to 34 days, the infected mosquitoes were again exposed to a normal rhesus but only three fed. The result was completely negative. The animal did not develop fever, did not circulate virus and failed to show immunity after a 30-day observation period. Only 13 *Sabethes* were available for mouse inoculation. Virus was not recovered in infant mice from any of them.

Experiment II

In the second transmission experiment (see Table 1) the local C-10 strain of yellow fever virus in its second monkey passage was used. Two lots of *S. chloropterus* were allowed to feed on a normal rhesus monkey, one on the 3rd day after subcutaneous injection, when the titer of circulating virus was $10^{-7.2}$, and the other on the 4th day when the titer was $10^{-5.3}$. The mosquitoes fed well. Specimens found dying or recently dead during the subsequent 27 days were used for mouse injection. (See mosquito lots 1 and 2 in Table 2.) Then all survivors of both lots were combined and allowed to feed on a normal young *Macaca mulatta*. This animal was exposed to the bites of the infected mosquitoes during a 3-day period after an extrinsic incubation period of 27 to 30 days. A total of 25 fed. As the monkey remained afebrile and apparently healthy during the succeeding week, it was re-exposed to the bites of the same mosquitoes which had previously engorged, the extrinsic incubation period at this time being 34 to 37 days. Fourteen mosquitoes refed. The rhesus began to circulate virus 5 days later and continued to show viremia for 3 days. It was febrile 6 days after the last exposure

TABLE 1

Results of attempted transmission of yellow fever virus to monkeys by bite

Virus strain	Titers of circulating virus in infective meals	Extrinsic incubation period (*days*)	Monkey used for bite transmission	Number of mosquitoes refeeding	Results
Experiment I					
Trinidad #4202	$10^{-5.2}$, $10^{-5.8}$ and $10^{-4.3}$*	32–34	Rhesus #1	3	Negative
Experiment II					
C-10	$10^{-5.3}$ and $10^{-7.2}$†	28–29	Rhesus #2	25	Positive‡
		35–36		14	
C-10	$10^{-5.3}$ and $10^{-7.2}$	42–43	Rhesus #3	13	Positive
Experiment III					
C-10	$10^{-6.4}$	29	Black spider #1	10	Negative
C-10	$10^{-6.4}$ and $10^{-6.5}$†	37–38	Black spider #2	14	Negative
C-10	$10^{-6.4}$	42	Black spider #3	8	Negative
C-10	$10^{-6.4}$ and $10^{-6.5}$	53–54	Black spider #4	8	Negative

* Pool of mosquitoes fed on 2nd to 4th days after inoculation of source monkey.

† Pool of mosquitoes fed on 3rd and 4th days after inoculation of source monkey.

‡ Time of infection uncertain as animal was exposed more than once.

TABLE 2

*Results of inoculation of infant mice with suspensions of individual mosquitoes or small pools**

Mosquito lot	Titer of virus in infective meal	Total numbers and percentages positive of mosquitoes according to weeks after feeding*									
		1–2 weeks		3–4 weeks		5–6 weeks		7–9 weeks		Totals	
		Tot.	% pos.	Tot.	% pos.	Tot.	% pos.	Tot.	% pos.	Tot.	% pos.
Experiment II											
Lot 1	$10^{-7.2}$	31	(16–26)†	5	(40–60)	—	—	—	—	36	(20–30)
Lot 2	$10^{-5.3}$	65	(1–6)	20	(5–25)	—	—	—	—	85	(3–14)
Lots 1 & 2	$10^{-5.3}$ and $10^{-7.2}$	—	—	—	—	19	(16–80)	12	(58)	31	(32–74)
Experiment III											
Lot 1	$10^{-6.4}$ and $10^{-6.5}$	32	(34)	18	(44)	8	(62)	19	(68)	77	(48)
Totals		128	(13–18)	43	(26–37)	27	(30–80)	31	(64)	229	(25–37)

* Data from experiment I are not included as all results were negative.

† Pools were included. Percentage positive depends on whether one or all mosquitoes in the pool are considered positive.

and showed irregular temperature rises, between 102.6° and 104.6° F., for a period of 5 days. It did not die, however. The blood possessed a high titer of protective antibodies 28 days after the first exposure, 19 days after the last. Negative reactions had previously been obtained with blood drawn prior to exposure and on the 14th day after the first exposure. Unfortunately, it cannot be determined definitely when the monkey acquired its infection, but it seems highly probable that this occurred during the second exposure and that the prolongation of the extrinsic incubation period was the crucial factor.

A second *Macaca mulatta* was then exposed to the bites of these same *S. chloropterus* for 1 day, after an extrinsic incubation period of 43 to 44 days. Thirteen engorged. This animal did not develop fever but became prostrate and died 7 days later when it was being prepared for a second exposure. Virus was recovered from its blood at this time. Thus, transmission was successfully effected by *S. chloropterus* to both rhesus monkeys under experimental conditions.

In the course of this experiment, 152 mosquitoes were used for mouse inoculation. Two- to 3-day-old mice were employed exclusively, 0.02 ml of the inoculum being delivered intracerebrally. Mosquitoes were triturated for injection either singly or in small pools of less than five. The use of some pools prevented, of course, the exact assessment of positive results. Examination of Table 2 will show that a positive virus isolation in infant mice depends on two factors: (1) the length of the extrinsic incubation period and (2) the titer of virus in the infective meal. After an extrinsic incubation period of 1 to 4 weeks, 20 to 30% of the mosquitoes (depending on whether only one or all of the mosquitoes in the positive pools are counted as positive) which had engorged on blood with a titer of $10^{-7.2}$, contained sufficient virus to pass the threshold of infection in infant mice; whereas, only 3 to 14% of those fed on blood with a titer of $10^{-5.3}$ demonstrated this capacity. A definite rise in the proportion of mosquitoes containing sufficient virus to infect infant mice may be observed in all groups as the extrinsic incubation period becomes more prolonged.

Experiment III

In the third experiment *S. chloropterus* were fed on a *Macaca mulatta* on the third and fourth days after subcutaneous injection with the C-10 strain of yellow fever virus, when the blood titers were $10^{-6.4}$ and $10^{-6.5}$, respectively. (See Table 1.) Juvenile black spider monkeys, captured in the wild state and housed at the Laboratory, were used instead of rhesus for bite-transmission attempts. Four of these *Ateles* were exposed to the attacks of the mosquitoes after incubation periods of 29, 37 to 38, 42 and 53 to 54 days, respectively.

Between eight and 14 mosquitoes engorged on each animal. However, no transmission was effected. None of the spider monkeys showed fever or any other symptom, circulated virus or developed protective antibodies. Other data accumulated in previous work indicate that Panama's black spider monkey has a high natural resistance against this disease.

A total of 77 mosquitoes was used for intracerebral injection of infant mice. All mosquitoes were ground and injected individually in order to obtain exact figures on the number of positives. A total of 37 (48%) contained sufficient virus to pass the threshold of infectivity of infant mice. (See Table 2.) The virus titers of the two infective meals were so closely similar that no attempt to classify the insects according to this criterion has been made. Again we may observe a steady increase in the proportion of positives with lengthening of the extrinsic incubation period; *i.e.*, 34% of 32 after 1 to 2 weeks of incubation and 68% of 19 after 7 to 9 weeks. Minimal quantities of virus were present, however; seldom sufficient to produce infection in all the mice of the inoculated groups. Four of the more strongly positive specimens were given a further 10-fold dilution after storage in CO_2 ice, and injected into infant mice, but none was able to infect all mice in this higher dilution and one failed to infect any. It is thus obvious that, even under the most favorable of circumstances, *Sabethes chloropterus* harbors only traces of virus.

DISCUSSION

An experiment of this kind always includes a number of uncontrollable variables which render accurate evaluation difficult. Certain factors entering into the complex have been clarified, however. It is now certain that *Sabethes chloropterus* may harbor small quantities of virus for life and transmit by bite. It is, nevertheless, by no means as efficient a vector as *Haemagogus*. We have obtained bite transmission only when the animal was attacked by a relatively large number of mosquitoes, whereas with *Haemagogus mesodentatus mesodentatus* transmission was readily obtained by the bite of a single specimen. There is also considerable individual variation in the capacity to transmit among the members of even a long-colonized population of *Sabethes*. Further study is required to determine what influence, if any, adaptation to colony life may exert.

In order to obtain transmission by this species there are certain requirements, namely:

1) an appropriate virus strain; 2) a high titer of virus in the infective meal; 3) a prolonged extrinsic incubation period; 4) a highly susceptible host.

The vector potentialities of *Sabethes chloropterus* have particular importance because this mosquito is long-lived and survives in fair numbers over the dry season. If it could harbor virus during this period and later transmit by bite, it would provide a solution to the perplexing problem of the survival of virus after the rains stop. Galindo and Trapido have been the principal advocates of this theory which was advanced in publications made in 1950, 1951 and 1956,[2, 3, 4] on epidemiological grounds. Other supporting evidence for the possible importance of *S. chloropterus* as a vector of yellow fever consists in the fact that this species has been found infected in nature. Shannon, Whitman and Franca[7] isolated yellow fever from a mixed lot of *Sabethoides*, *Limatus*, *Wyeomyia*, and *Trichoprosopon* (in which *S. chloropterus* was included), but it is not known which of the species was infected. More recently Rodaniche and Galindo[5] and Rodaniche, Galindo and Johnson[6] have reported the isolation of yellow fever virus from pure pools of *Sabethes chloropterus* captured in Guatemala and Panama during periods of epizootic activity in those countries.

In 1957 an attempt was made to recover virus from this species over the dry season. Virus had been active at Cerro Azul in Panama, near the end of the rainy season, in August, September and October, 1956. It was recovered from mosquitoes seven times during this period, including twice from *Sabethes chloropterus*. Captures were continued during the dry months, a total of 243 specimens being obtained (79 in January, 48 in February, 38 in March, 46 in April and 32 in May). However, no yellow fever virus was recovered.

SUMMARY

Confirmation is provided of previously reported experimental transmission of yellow fever virus by the bites of *Sabethes chloropterus*. This mosquito is a relatively inefficient vector, however, and requires a suitable virus strain, a high titer of virus in the infective meal, a prolonged extrinsic incubation period and a highly susceptible host, to be successful.

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SPECIES CROSSING IN *ONCOMELANIA*[1]

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During the past 6 years this laboratory has studied the biology of the four recognized species of the snail, *Oncomelania*, occurring in the orient. This was done to provide basic data which are helpful in organizing a program for the eradication of these snails in an attempt to control schistosomiasis.

As part of the study dealing with reproduction it seemed important to determine whether or not interbreeding can occur among some or all of the four recognized species, and if so, if the hybrids are fertile. This paper presents the findings and gives more complete data than those reported earlier (Wagner, *et al.*, 1957). DeWitt's (1954) finding that the geographical strains of the parasite, *Schistosoma japonicum*, do not develop in all of the four species of the snail, was of further interest in making the species-crossing tests. His work showed that the Philippine strain of the parasite develops only in the Philippine snail, (*O. quadrasi*), the Chinese strain develops only in the Chinese snail (*O. hupensis*), while the Japanese strain develops in both the Japanese snail (*O. nosophora*) and the Chinese snail. The Formosan strain develops in all of the snails except the Chinese species, (*O. hupensis*). How this infective picture would be altered if fertile hybrids of these snails were introduced to the snail population might prove of practical value.

Barbosa, *et al.*, (1956a) obtained successful crossing between species which are placed in two genera. They crossed albino *Australorbis glabratus* from Recife, Brazil with pigmented *Biomphalaria boissyi* from Cairo, Egypt. Fertile hybrids resulted from the cross and Mendelian segregation was observed, with all F_1 progeny being pigmented. F_2 offspring had a 3:1 ratio of pigmented to albino snails.

[1] This work was conducted under the sponsorship of the Commission on Parasitic Diseases, Armed Forces Epidemiological Board, and was supported by the Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-307.

Appreciation is expressed to Mr. Dale Inaba for technical assistance given.

Barbosa (1956b) was also successful in crossing two species of the same genus, *Tropicorbis centimetralis* from Brazil, and *T. chilensis* from Chile.

METHODS AND MATERIALS

Very young snails of the four species hatched in culture dishes were individually isolated in 60-mm Petri dishes prepared as rearing containers. The snails were allowed to grow to adult size, after which they were sexed by the method of Wong and Wagner (1954). A male of one species was placed in a 100-mm rearing dish with a female of another species as per the crossings indicated in Table 1. Reciprocal crossings of the sexes of each species combination were made. All 12 possible crossing combinations were tested in interbreeding the four species. A total of 131 matings of these combinations were made.

The rearing dishes were maintained in culture rooms kept at a nearly constant temperature of 26° C and provided with a constant light source. (For details on culture methods used, see Wagner and Wong, 1956.) The dishes were periodically examined for the presence of eggs and young snails. When young were found they were transferred to new culture dishes and observed for the production of F_2 generations.

The crossings between *O. nosophora*, *O. formosana*, and *O. quadrasi* were made during the period April, 1954, to January, 1957, and these three species were crossed with *O. hupensis* during the period September, 1956, to August, 1958.

RESULTS

Hybrid snails resulted with varying degrees of success in all of the 12 possible cross matings made in the four species of *Oncomelania*. The per cent successful crossings obtained ranged from 5.5 to 80.8 (Table 1). The number of offspring in the test varied from an average of 51 per pair of snails that produced young, to one snail for a pair. (In *O. hupensis* ♂ + *O. formosana* ♀ and *O. quadrasi* ♂ + *O. formosana* ♀, respectively.) A small sampling of F_1 offspring

TABLE 1

Oncomelania *species crossing mating combinations with F_1 and F_2 results*

Oncomelania crossing combinations	No. of matings	Successful crossings		Total number F_1 (hybrids) seen	F_2 obtained
		No.	%		
O. nosophora ♂ + *O. quadrasi* ♀	4	3	44.4	48	F_2
O. nosophora ♀ + *O. quadrasi* ♂	5	1		8	
O. nosophora ♂ + *O. formosana* ♀	5	2	45.4	88	F_2
O. nosophora ♀ + *O. formosana* ♂	6	3		37	
O. quadrasi ♂ + *O. formosana* ♀	7	2	33.3	2	F_2
O. quadrasi ♀ + *O. formosana* ♂	5	2		5	
O. hupensis ♂ + *O. quadrasi* ♀	11	2	22.2	20	F_2
O. hupensis ♀ + *O. quadrasi* ♂	7	2		2	—
O. hupensis ♂ + *O. nosophora* ♀	26	1	5.5	12	F_2
O. hupensis ♀ + *O. nosophora* ♂	29	2		10	—
O. hupensis ♂ + *O. formosana* ♀	13	12	80.8	615	F_2
O. hupensis ♀ + *O. formosana* ♂	13	9		335	F_2

for sex ratios gave an indication that there is a normal sex balance, *i.e.*, a slight excess of females. Crossings involving *O. formosana* were generally the most successful, while those of *O. nosophora* were rather poor. This agrees with laboratory observations made in reproduction for each of the four species of snails. Arranged in the order of most to least prolific reproduction within their own species they are: *O. formosana*, *O. hupensis*, *O. quadrasi*, and *O. nosophora*. See Table 2 for "species crossing" and "intraspecific" matings compared for per cent successful crossing and number of young or eggs produced.

Hybrids resulting from the crossings were mated and found to be fertile. In the first set of crossings, involving *O. nosophora*, *O. quadrasi*, and *O. formosana*, offspring resulting from the matings were placed together for each cross, and in all cases F_2 generations were obtained. In the second set of crossings (*O. hupensis* with the other species) hybrid snails resulting from each cross, including the reciprocals, were mated to test for hybrid fertility. F_2 generations resulted in the *O. hupensis* male + *O. quadrasi* female cross. Insufficient numbers of young were obtained in the reciprocal cross to make a mating. F_2 offspring were obtained in the *O. hupensis* male, *O. nosophora* female cross. No matings could be made in this reciprocal cross due to insufficient numbers resulting in the cross and due to a high mortality rate in the few hybrids. F_2 snails resulted from *O. hupensis* male + *O. formosana* female hybrid matings and in the reciprocal cross hybrid matings. This was the most successful cross in all the matings made in respect to the number of F_1 and F_2 generations produced in the test.

An additional successful cross involved a virgin female *O. nosophora* mated with a male hybrid resulting from an *O. hupensis* + *O. formosana* cross. The number of young obtained in this cross was about the same as in a normal (non-cross) mating.

The hybrids resulting from the various species crossings vary considerably in their characteristics. The bright yellow "eyebrows" with coarse, compact pigment (characteristic of *O. quadrasi* and *O. formosana*) appeared to be present in the hybrids more frequently than the light yellow "eyebrows" with fine granules (characteristic in *O. nosophora* and *O. hupensis*) in matings involving these two groups of snails. However, progeny having the type of "eyebrow" typical for the *O. nosophora* and *O. huypensis* parents appeared, as did a few which had an intermediate type of "eyebrow," especially in respect to granules organized in one mass or separated into two masses.

The prominent ridges occurring on the shell of *O. hupensis* appeared infrequently in hybrids resulting from crosses with the other three species of *Oncomelania*. Striations were present more frequently and occurred in about the same number and position as the ridges but instead of the ridges. In most of the hybrids where ridging was present, the ridges were not of the prominent type.

The sizes of the hybrids ranged from the extremes represented by the parents of the crosses

TABLE 2

Species-crossing matings and two intraspecific matings compared

Species mated	Successful: unsuccessful matings	Successful mating (%)	Total young or eggs (e)	Average young or eggs per female	Mating period (months)
nosophora + *quadrasi*	4/9	44.4	56	14.0	34
nosophora + *formosana*	5/11	45.4	125	25.0	34
quadrasi + *formosana*	4/12	33.3	7	1.7	34
hupensis + *quadrasi*	4/18	22.2	22	5.5	24
hupensis + *nosophora*	3/55	5.5	22	7.3	24
hupensis + *formosana*	21/26	80.8	950	45.2	24
1. *formosana* + *formosana*	9/10	90.0	158 (e)	17.6	4
2. *formosana* + *formosana*	5/7	71.4	610	122.0	9
hupensis + *hupensis*	*				
1. *quadrasi* + *quadrasi*	11/15	73.3	827 (e)	75.2	11
2. *quadrasi* + *quadrasi*	15/17	88.2	2273	151.5	12
1. *nosophora* + *nosophora*	10/19	52.6	320 (e)	32.0	7
2. *nosophora* + *nosophora*	10/17	58.8	1551	155.1	9

* Placed in this position from stock rearing results observed.

with intermediate sizes occurring in some. The size of the larger parent snail was attained by most of the hybrids.

A few hybrids showed a "mixture" of characteristics. A snail resulting from the *O. nosophora* female + *O. hupensis*, *O. formosana* hybrid male crossing had prominent ridging on the sixth whorl while the other whorls were smooth. A few hybrid snails from crosses involving *O. hupensis* had ridges on the lower side of the shell and not on the sides or top. In some the "eyebrows" were so lightly pigmented as to appear absent.

DISCUSSION

It is not the purpose of this paper to deal with the taxonomic problem of the four species of *Oncomelania* found in the four geographical situations in the Orient (Abbott, 1948a, also gives *O. nosophora slateri* Bartsch as a valid subspecies in China). However, it is of interest to comment on the subject. In Hubendick's (1954) review on viewpoints of species discrimination in medically important snails, he points out that species are usually determined on a morphological basis, but interbreeding tests, if feasible, would be more valid. He also suggests chromosome morphology and serological diagnostics as means of species discrimination.

Even if it were feasible to determine the taxonomic status of medically important snails by these means, the present authors agree with Hubendick that it is questionable that a change in the present taxonomic status would serve a good purpose. Apparently as a result of their investigations, Barbosa and Hubendick agree in considering the African *Biomphalaria* and the New World *Australorbis* congeneric, but for the sake of the clinician and the Public Health worker, they should be left as they are.

If laboratory interbreeding of the four species of *Oncomelania* can serve as a criterion of the validity of a biological species, these snails should probably be considered subspecies. However, it may be prudent to apply the same solution as that suggested by the workers quoted above, and leave the nomenclature as it is.

It would be of interest to determine whether or not *Pomatiopsis* will interbreed with *Oncomelania*, as Abbott (1948b) makes reference to the fact that the North American species, *Pomatiopsis lapidaria*, has a close morphological similarity to *O. nosophora* (Robson) and that "their common ancestor's range extended across the old Bering Sea connection."

SUMMARY

The four species of *Oncomelania*, namely, *O. quadrasi*, *O. formosana*, *O. nosophora*, and *O. hupensis* of the orient were successfully interbred

in all 12 possible species-crossing combinations, including the male and female reciprocal matings. Fertile hybrids resulted from all the crossings. In addition, a male hybrid resulting from an *O. formosana* + *O. hupensis* cross was crossed to a female *O. nosophora*.

The hybrids from the species crossings had characteristics which were intermediate to those of the parents as well as representing those of the parents. Although no dominant or recessive characters were evident, the bright yellow, coarse, compact "eyebrows" of *O. quadrasi* + *O. formosana* appeared more frequently than the light yellow, fine "eyebrow" pigment of the other species. The prominent shell ridges of *O. hupensis* appeared infrequently in the hybrids. The most successful crossing was obtained in the *O. hupensis* + *O. formosana* matings.

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BOOK REVIEWS

Drugs: Their Nature, Action and Use, by HARRY BECKMAN. 728 pages. Philadelphia, W. B. Saunders Company. 1958. $15.00

This new textbook has been written "for the undergraduate medical student . . . to prepare for the pharmacologic aspects of the practice that lies ahead." For this purpose it should serve admirably. Of special interest in this connection are the chapters which deal with broad concepts of pharmacology and the relation of pharmacology

to other basic medical sciences. The book can be recommended equally to physicians, pharmacologists and those in the allied medical sciences as an up-to-date and comprehensive source of information about current therapeutic agents.

Drugs are arranged in broad groups on the basis of the types of effects they produce. In the discussions of individual agents, both generic and trade names are given and data are included on source and nature (including structural formula), clinical effects, mode of action, metabolism, dosage and toxicity. Lists of references are provided at the end of each section. Though these are not meant to be exhaustive, they should prove adequate as guides to the pertinent literature. References to many recent publications are included; these attest to the thoroughness with which the author has assessed current developments in this ever-changing field.

CARL C. SMITH

A Guide to Human Parasitology (Blacklock & Southwell), by T. H. DAVEY (Rev.). Sixth edition, 222 pages, illustrated. Baltimore, Williams & Wilkins Company. 1958. $7.00.

The book is written for the medical practitioner and especially to be of help to those who have no laboratory near at which parasitological diagnoses can be made.

The material is divided into 22 chapters. The first four chapters are concerned with a general statement, the microscope, examination of material (principally protozoan), and general remarks on parasitology.

Chapter V discusses spirochetes and illustrates the pattern for presenting the technical information on the parasites, *i.e.*, a general statement, the scientific name, geographical distribution, habitat, morphological characters, life history, pathogenicity, diagnosis, and prevention. Treatment is not discussed.

There are six chapters on the protozoa. Brief descriptions of *Isospora* and *Toxoplasma* have been introduced. A diagram of the life history of the malaria parasite is included, incorporating the new knowledge on the tissue phases.

Chapter XII introduces the helminths. Chapter XIII again considers examination of material, principally helminths, but some reference is made to the previous chapter dealing with examining of material for protozoa. It appears to the reviewer that the combining of these two chapters would make for efficiency.

The remaining chapters deal with the helminths. Finally there are 16 pages of diagrams illustrating life histories of helminths grouped according to the mode of entry into the human body.

There are three full-page color illustrations which are well done. These plates are not placed in the chapters concerning the parasites they illustrate. In fact, one plate showing malaria parasites is 20 pages removed from another plate showing malaria parasites in thick smears.

The author's language is clear and the information is interestingly presented. The book appears to be relatively free of errors of fact and of typesetting and is attractively bound. The current edition under the capable editorship of Dr. T. H. Davey should continue to serve well, as did its predecessors, the purpose for which it was written.

MARTIN D. YOUNG

Tuberculosis in White and Negro Children, Volume I: The Roentgenologic Aspects of the Harriet Lane Study, by JANET B. HARDY, M.D.C.M., **Volume II: The Epidemiologic Aspects of the Harriet Lane Study,** by MIRIAM E. BRAILEY, M.D. First edition, 122 and 103 pages, respectively, illustrated. Cambridge 38, Mass., Published for The Commonwealth Fund by Harvard University Press. 1958. $7.50 and $4.50, respectively.

These two volumes constitute the long-awaited report on the Harriet Lane Study of tuberculosis in children. Over 1000 children were entered in this long term study between 1928 and 1944; the patients were followed until the study terminated in 1950. The first volume is essentially an atlas of roentgenograms demonstrating very well many forms of tuberculosis seen in children, with the addition of films illustrating differential diagnosis of tuberculosis and other pulmonary conditions. The natural course of tuberculosis is shown by serial roentgenograms sometimes supplemented by bronchoscopic findings or illustrations from material obtained postmortem. The captions contain relatively little clinical information. There is a useful section on anatomy of the lung in relation to interpretation of chest x-rays and a chapter on bronchoscopy and bronchography.

The second volume consists of an analysis of the records of the Harriet Lane Study, mainly concerned with the prognosis of primary tuberculous infection in children and with the risk to the survivors of later developing "reinfection"-type pulmonary tuberculosis. Dr. Brailey, who was the director of the study from 1932 through 1941, is eminently qualified for the task of interpretation of this material and has presented it in a very interesting and readable form. This is a basic book for workers concerned with any aspect of tuberculosis in children and should continue to be of value as reference material for many years. A closer integration of the material in the two volumes would have been desirable.

EDITH M. LINCOLN

Diagnostic Medical Parasitology, by EDWARD K. MARKELL, Ph.D., M.D., AND MARIETTA VOGE, M.A., Ph.D. first edition, 276 pages, illustrated. Philadelphia, W. B. Saunders Co. 1958. $7.00.

The subject matter of this book is based primarily on the material in parasitology included, with information on other infectious agents, in the course of Infectious Diseases given in the School of Medicine, University of California at Los Angeles. The method of presentation is according to organs or organ systems of the patient. Following two introductory chapters, the first of which is in a sense a continuation of the Preface and the second a succinct but comprehensive consideration of Parasites, Parasitism and Host Relations, the third chapter provides instruction for examination of the feces for discovery of intestinal protozoa. Chapter four presents descriptions of the six species of intestinal amebae reported from man, the intestinal flagellates, the two species of human *Isospora*, and *Balantidium coli*, followed by a brief description of the protozoa of the mouth. Chapter five considers in some detail the Intestinal Helminths, namely the trematodes, cestodes and roundworms.

After brief instruction on Procedures in Examination for Blood Parasites, there is a well-rounded presentation of Malaria (Chapter 7), followed by a consideration of Hemoflagellates and Filariae. Chapter nine deals with the genitourinary parasites, *Trichomonas vaginalis*, *Schistosoma haematobium* and *Dioctophyma renale*. The Tissue Parasites are considered in Chapter ten, including *Toxoplasma gondii*, *Pneumocystis carinii*, the cysticercus of *Taenia solium*, the hydatids of *Echinococcus granulosus* and *E. multilocularis*, the cenurus of *Multiceps*, the sparganum of *Diphyllobothrium* spp., *Onchocerca volvulus*, *Dracunculus medinensis*, *Trichinella spiralis* and the agents of visceral larva migrans. Chapter 11 discusses Arthropods and Human Disease, including those as parasites and as transmitters of disease-producing microörganisms. This is followed by helpful information on Pseudoparasites and Pitfalls, and two chapters on diagnostic methods and procedures. There are two pages of references cited in the text and a short subject index.

The text is factual, up-to-date and clearly presented, but lacks coordination. The reader marvels that so much material has been telescoped into 276 pages, when approximately 30 per cent of the space is occupied by illustrations. These merit special commendation, both the diagrammatic line-and-stipple drawings and the remarkably fine photos and photomicrographs, most of the latter originals by Zane Price. Five color plates on the malaria parasites add to the value of this chapter. It is unfortunate that the drawing intended to show the differential characters between the rhabditoid larvas of *Strongyloides* and the hookworms (Fig. 59, p. 116) does not include the genital primordia, the most readily observed differential character. Likewise, failure to mention the work of Vogel (1955, 1957) on *Echinococcus multilocularis*, and of Beaver and associates (1952–1956) in first demonstrating the etiology of visceral larva migrans is regrettable.

Since the authors have oriented their presentation towards parasitism and organs and organ systems, they have refrained from presenting any systematic classification of the protozoa and helminths. This has its drawbacks, and from a physiopathological viewpoint has at times led to serious difficulties, as, for example, separation of *Schistosoma mansoni* and *S. japonicum* from *S. haematobium*, since the fundamental early phase of the cycle of all three species in man is essentially the same and even the mature host-parasite relations of *S. haematobium* are frequently not organotropically as distinct from those of the other two species as the reader might be led to conclude. Furthermore, too little emphasis is placed on systemic manifestations of parasitic diseases. While laboratory diagnosis is well presented, the reviewer believes that the attempt of the authors to relate the various infections to symptoms has not been achieved.

The format of the book is pleasing, the paper is excellent for illustrations and text, and typographical errors are unusually few for a first edition. The volume is recommended as a valuable laboratory guide for medical students and laboratory technicians.

ERNEST CARROLL FAUST

Antisera, Toxoids, Vaccines & Tuberculins, by H. J. PARISH, M.D., F.R.C.P., D.P.H., Clinical Research Director, Wellcome Foundation, London. Fourth edition, 296 pages, illustrated. Edinburgh & London, E. & S. Livingstone, Ltd., (Williams & Wilkins, exclusive U. S. agents). 1958. $7.00.

The fourth edition of this handbook maintains and passes the standards of its predecessors. The revision has been extensive. Obsolescent material has been eliminated or reduced and data on, and discussions of, new developments expanded. The latter is particularly true of the material on immunization against viruses.

The book is written for practicing physicians, senior students and other advanced readers desiring a concise summary of important current opinions, practices and data concerning the matters dealt with. It is not a textbook, or refer-

ence work for researchers. It deals primarily with the production, standardization, indications pro and con, use, regulation, control, etc., of immunological materials and gives, in concise form, reviews of the underlying principles. It is directed to the British medical reader and uses British practices and experience as a basis for discussion. This furnishes material for debate on such topics as the value of combined antigens; alum-precipitated antigens vs., fluid toxoids; bacillary vaccines vs., pertussis and diphtheria; use of antigens such as alcohol-treated enteric vaccines; and so on. There are sound discussions on the use and value of B.C.G., with limited and not-too-encouraging data on the Vole-bacillus vaccine, and a chapter on polio vaccine. It is of interest that in the British vaccine the Mahoney Type I strain is replaced by the Enders Brunhilde strain and that vaccine became available in 1958 for all children under 15, pregnant women, and persons at special risk.

Bibliographical citations are mostly in the text and, while not too numerous, are pertinent and up to date. There is a list of related reports, standard reference works and texts, and an annotated list, with dates, of important discoveries and events in the development of immunology, beginning with Lady Wortley Montague in 1721. The numerous portraits and other illustrations (some in color) contribute much to the value of this useful and informative volume.

M. FROBISHER

World-Atlas of Epidemic Diseases, Edited by Ernst Rodenwaldt and Helmut J. Jusatz, under the Sponsorship of the Heidelberger Akademie der Wissenschaften. Second of four issues in the third part of the series. 32 pp., 10 maps. Hamburg: Falk Verlag.

The second installment of the third part of the atlas of epidemic diseases ought to be specially attractive to those interested in the morbidity of infectious conditions since, apart from a map illustrating the thermic sultriness zones (*Schwüle-Zonen*) of the world and a somewhat disappointing general map of Asia, it deals with the recent incidence of the following important transmissible affections: (i) cholera in Asia 1931–1955; (ii) bacterial dysentery (*S. dysenteriae* 1) in Asia 1900–1954; (iii) global distribution of diphtheria 1934–1953; (iv) plague pandemic of the 20th century; (v) poliomyelitis in all countries for which information was available during the period 1947–1955; (vi) global distribution of smallpox 1949–1955; (vii) world distribution of trachoma 1930–1955 and (viii) global distribution of filariasis 1955.

Exhaustively dealing with the recent incidence of cholera, Rodenwaldt points out that "since the turn of this century cholera has continually lost ground, even in its original 'native' land, India and Indochina." As is generally known, with one exception (Egypt, 1947) this disease has not been able to gain a foothold outside Asia since 1930. In his opinion, this inroad of the infection was quickly brought under control through vaccination, a method which, as he emphasizes, was also found most effective for the control of the large pilgrimages in India.

Discussing the problem of bacterial dysentery in Asia, Seeliger refers to the fact, known also to all other competent and impartial observers, that the incidence of this infection is rather underrated in favor of that of amebic dysentery and stresses that, moreover, no information on the occurrence of dysentery is available for large parts of the continent. There is, however, not the least reason to doubt that bacterial dysentery is a most serious health problem in all parts of Asia.

In his study on the global incidence of diphtheria, Kanter maintains that "the tropics, with their less virulent strains, are the reservoir of the disease. If these are brought to temperate climates, the infection assumes a more serious form."

Raettig, commenting on the occurrence of plague manifestations in the 20th century, points to the constant and marked regression of this disease within recent years but maintains with great reason that the infection remains entrenched in several wild-rodent foci which, therefore, must be watched with greatest care. Sharing the opinion of all workers familiar with the problem of plague, he declares, however, that, if signs of tendency for a spread of the infection from such foci are recognized early, "it should be possible with the present means of plague control and with energetic international cooperation, to prevent any further plague catastrophes."

Aptly discussing the problem of the global distribution of smallpox, Henneberg stresses that "As long as still about 687 million people, *i.e.*, more than the fourth part of mankind, live in smallpox-infected regions or in close vicinity with such, there is a latent danger for the entire human race which . . . can be encountered only through inoculation and other defensive measures."

The great desirability of exterminating the permanent haunts of the disease through united efforts is therefore obvious.

The important conclusions reached by Siebeck after a consideration of the global distribution of trachoma are that the results achieved thus far through international cooperation "are encouraging, although they are limited in many

countries by the terrible social conditions. The further success of trachoma control depends to a great extent on how far it is possible to raise the standard of living in the vast trachoma regions and to change the way of life of the people."

"As long as unrest and turmoil continue in Asia, only a cautious prognosis can be made for the further developments. In places such as Europe, America and Australia, however, where a peaceful development can be expected, there is every reason to believe that the disease will recede further in the course of the next decades."

Concluding a particularly well documented study on filariasis, Erhardt and Wellensiek state that even though human filarial infections are still very widespread in the tropics and subtropics, "thanks to the modern means of controlling the intermediary hosts, they are on the retreat everywhere, and are already exterminated in Europe and North America and are disappearing in Australia. In addition, infections in man can be more or less successfully influenced by surgical or therapeutic methods with new medicaments. . . . There is, then, good reason to hope that filarial infections, the scourge of the warm countries, will progressively lose their sting in the not too distant future."

R. POLLITZER

TRANSACTIONS OF THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

Seventh Annual Meeting

Miami Beach, Fla., November 5–8, 1958

OFFICERS

President—Donald L. Augustine
President-Elect—Lewis W. Hackett
Vice-President—Paul C. Beaver
Secretary-Treasurer—Rolla B. Hill
Editor—Martin Frobisher

COUNCILORS

M. C. Balfour
L. A. Stauber
J. M. Henderson
P. F. Russell
T. H. Weller
W. C. Reeves
J. A. Scott
J. E. Smadel

FORMER PRESIDENTS OF THE SOCIETY

Martin D. Young	1952
William A. Sodeman	1952–53
Frederick J. Brady	1953–54
William A. Taliaferro	1954–55
Asa C. Chandler	1955–56
Justin M. Andrews	1956–57

MINUTES OF THE ANNUAL COUNCIL MEETING

The annual business meeting of the Council was held on November 5, 1958, with President Augustine in the Chair. All Officers and Councilors were present with the exception of M. C. Balfour and J. M. Henderson, both of whom were unavoidably absent.

The minutes of the sixth annual meeting, held in Philadelphia, October 30–November 2, 1957, were approved as published in the *Journal* in March, 1958.

The Secretary's report was presented as follows:

This report covers the fiscal year, from October 1, 1957 to September 30, 1958.

Annual Meetings. The sixth annual meeting of the Society was held jointly with the American Society of Parasitologists, in Philadelphia on October 30–November 2, 1957. Almost 500 persons registered for the meeting. The Co-Chairmen of the Local Committee in charge of arrangements were Drs. C. Brooke Worth and George L. Graham.

The printed programs for the 1958 annual meeting were mailed to all members on September 6.

The Council has agreed to hold the 1959 meeting in Indianapolis, October 25–28.

Membership. As of September 30, the Society had enrolled, 1,086 Active Members in good standing, plus 2 Life Members, 11 Emeritus Members and 12 Honorary Members, a total of 1,111, and an increase of 13 over the previous year. In addition, there were 58 members in arrears of dues for 1958, 36 in arrears for 1957 and 1958; and 26 whose dues were unpaid for 1956, 1957 and 1958, a total of 120 members whose dues are unpaid for one to three years. Adding these to the 1,111 in good standing, we have a total membership of 1,231.

Included in the above total are 70 new members, whom we welcome into the Society. There were 64 new members elected in 1957.

With much regret, we record the death of the following valued members:

Ralph F. Allen
Edwin S. Gault
William W. Hersohn
Walter G. Humbert
Charles R. Rein
Michael J. Takos

and Asa C. Chandler, who was our President in 1955–56.

Twenty-seven members submitted their resignations during the year, and 28 members, de-

linquent in dues for 1955, 1956 and 1957, were dropped from the rolls in accordance with Art. XI of our By-Laws.

Letters to the Council. On January 15, a proposal that publication of the Abstracts of papers presented at the annual meeting should be stopped, was submitted to the Council. This proposal was unanimously approved.

On the same date the Council approved the dates of October 25–28 for our 1959 meeting in Indianapolis. Dr. Max C. McCowen was appointed Local Chairman for this meeting.

On March 15, authorization was requested to sell our K bonds to a maximum of $2,500.00, to take care of an anticipated deficit in the current expenses of the Society for the year. The Council gave the necessary authorization, but no bonds were sold since by deferring certain payments we were able to close the year with a small cash balance.

On May 20, the Council was informed of several developments relating to Society business, including the receipt of a proposal by five members that the "Fellow" class of membership be abolished, through a Constitutional Amendment, as provided by Article V. The petition being in order, the Council approved submission of a ballot to the membership to allow them to vote on the proposal.

On June 10, the Council was informed of the plans for the 1958 annual meeting, along with minor items of Society business.

Other activities. The revision of the NEWS "free" mailing list, begun in 1957, was completed, with the elimination of some 300 names of persons who did not respond to the "continuance" slips included in two numbers of the NEWS. An equal number of new names was added to the mailing list, with a return slip included for the recipient to return if he wished the *News* to continue. The response was excellent, and the mailing list continues at around 2,000, this total naturally including the membership list. The Secretary's office keeps the stencils and addresses and prepares the envelopes for each issue of the *News*.

Under the contract with the International Cooperation Administration, whereby the latter underwrites membership in the Society for certain of its past and present trainees, two new members were admitted during the year, with a third person having presented his application.

In response to official invitations, the President appointed Dr. John F. Kessel to represent the Society at the inauguration of Dr. Norman H. Topping as President of the University of Southern California; and Dr. George H. Saunders as our representative at a dinner tendered to Dr. Edward I. Salisbury on his retirement as Medical Director of the United Fruit Company.

Activities in connection with the various Committees will be reported under the appropriate headings.

Respectfully submitted,
ROLLA B. HILL,
Secretary

The report of the Secretary was accepted as read.

The Treasurer's report was presented as follows.

The independent auditor's report is shown below.

The Officers and Councilors October 24, 1958
The American Society of
Tropical Medicine and Hygiene
Miami, Florida

Gentlemen:

I have examined the financial records of *The American Society of Tropical Medicine and Hygiene*, Miami, Florida, and *Tropical Medicine and Hygiene News*, Bethesda, Maryland, for the fiscal year ended September 30, 1958 and now submit as my report the exhibits, schedules and comments listed below.

SCOPE OF EXAMINATION

My examination was made in accordance with generally accepted auditing standards, and accordingly, included such tests of the accounting records and other auditing procedures as I considered necessary in the circumstances to enable me to render an independent accountant's opinion on the fairness of the representations in the statements showing the assets and liabilities of the organization at September 30, 1958, and its operating results for the fiscal year then ended. Auditing procedures employed included those set forth in the following paragraphs.

The bank balances were confirmed by direct communications with the depositories. All cash receipts and disbursements were traced through the bank accounts. All other current assets, with the exception of (1) the inventory of "Health Hints for the Tropics" included at a cost of $287.62 and (2) the account receivable of $1,311.68 due from Williams and Wilkins as *estimated* profit on 1958 issues of the *Journal*, were either computed by me or otherwise satisfactorily verified. The estimated profit from Williams and Wilkins for the previous fiscal period ended September 30, 1957 was $1107.04, but after publishing the final two issues the net profit for the calendar year 1957 amounted to only $27.57 for the publication of the full six issues. Therefore, the estimated profit

shown on the balance sheet under "Account Receivable" may be subject to considerable change by the end of the calendar year 1958 when the actual net profit is computed by the publishers.

The United States Savings Bonds in the amount of $20,198.00, stated at cost, were inspected at the Coconut Grove Bank and found to be in the name of the Society.

There were no changes in fixed assets during the fiscal year.

I made sufficient tests of the operating accounts to satisfy myself that accepted accounting practices had been followed and that the transactions for the fiscal year had been properly and consistently classified. As to the receipt of membership dues, it was not practicable under the scope of the engagement to extend the review beyond ascertaining that the receipts, as reported on the books, had been deposited in the Society's bank account.

ACCOUNTANT'S OPINION

In my opinion, the accompanying balance sheets, and related cash receipts statements, fairly present the assets and liabilities of *The American Society of Tropical Medicine and Hygiene*, Miami, Florida, and *Tropical Medicine and Hygiene News*, Bethesda, Maryland, at September 30, 1958, resulting from cash transactions, and the income collected and the expenses disbursed for the fiscal year then ended, and are presented on a basis consistent with that of the preceding year.

W. KEITH WHEELING,
Certified Public Accountant

EXHIBIT A

The American Society of Tropical Medicine and Hygiene
Miami, Florida
COMPARATIVE BALANCE SHEETS
September 30, 1958 and 1957
Assets

	September 30, 1958		*September 30, 1957*	
Current assets:				
Cash in bank (Exhibit B)	$1,913.22		$3,359.64	
Account receivable (Note 1)	1,311.68		1,107.04	
Interest receivable, U. S. Savings Bonds (Exhibit A, Schedule 1)	179.40	$3,404.30	179.40	$4,646.08
Investment assets:				
U. S. Savings Bonds, at cost (Exhibit A, Schedule 1) (Note 2)				
Series J (Maturity value $275.00)	$198.00		$198.00	
Series K (2.76%)	20,000.00		20,000.00	
	$20,198.00		$20,198.00	
Tropical Medicine and Hygiene News (Exhibit C)	2,629.50	22,827.50	1,828.73	22,026.73
Fixed assets:				
Office equipment		472.24		472.24
Total assets		$26,704.04		$27,145.05

Liabilities and Net Worth

Current liabilities:		
Editorial office expense for fiscal year due Dr. Hackett (Exhibit B, Schedue 1)		$2,010.70
Payroll taxes and withheld wages due	$45.00	
Deferred credits to income:		
Prepaid dues	96.00	61.50
Total liabilities	$141.00	$2,072.20

Net worth:				
Retained income				
Balances, September 30, 1957 and 1956	$24,600.61		$25,876.11	
Increase for fiscal years ended September 30, 1958 and 1957	1,490.19		(1,275.50)	
Balances, September 30, 1958 and 1957 (Note 3)	$26,090.80		$24,600.61	
Investment in office equipment	472.24	26,563.04	472.24	25,072.85
Total liabilities and net worth		$26,704.04		$27,145.05

Note 1. The account receivable represents the *estimated* profit from the 1958 publication of four issues of the *American Journal of Tropical Medicine and Hygiene* by the Williams and Wilkins Company.

Note 2. Interest on U. S. Savings Bonds, Series J, is available for current expenditures; interest on Series K Bonds is available for reviews in the *Journal* and for publishing abstracts of papers on the program.

Note 3. Of the $26,090.80 in retained income as of September 30, 1958, $3,314.34 is unappropriated as of that date, the remainder having been appropriated for:

Extra pages for the *Journal*	$1,904.38
Life membership fee of Dr. Charles A. Werner invested in U. S. Savings Bonds, Series J	198.00
Investment in U. S. Savings Bonds, Series K, the interest on which is available for reviews in the *Journal* and for publishing abstracts of papers on the program	20,000.00
Interest on Series K Bonds collected to September 30, 1958, less expenditures to date (see Exhibit A, Schedule 1)	674.08
Total appropriated retained income	$22,776.46

EXHIBIT A

Schedule 1

The American Society of Tropical Medicine and Hygiene

Miami, Florida

INVESTMENTS IN U. S. SAVINGS BONDS

September 30, 1958

Serial number	*Date of purchase*	*Cost*	*Maturity value*	*Accrued interest*
Series J				
Q 21413 J	6-29-54	$18.00	$25.00	
Q 21414 J	6-29-54	18.00	25.00	
Q 21415 J	6-29-54	18.00	25.00	
C 93631 J	6-29-54	72.00	100.00	
C 93632 J	6-29-54	72.00	100.00	
Total investment Series J		$198.00	$275.00	
Series K				
M 91038 K	4-14-53	$1,000.00	$1,000.00	$13.80
M 91039 K	4-14-53	1,000.00	1,000.00	13.80
M 91040 K	4-14-53	1,000.00	1,000.00	13.80
M 91041 K	4-14-53	1,000.00	1,000.00	13.80
M 195142 K	6-29-54	1,000.00	1,000.00	9.20
V 26490 K	6-29-54	5,000.00	5,000.00	46.00
V 26514 K	7-9-54	5,000.00	5,000.00	34.50
V 26515 K	7-9-54	5,000.00	5,000.00	34.50
Total investment Series K		$20,000.00	$20,000.00	$179.40
Total investments in U. S. Savings Bonds		$20,198.00	$20,275.00	

Notes:

(1) There were no changes in the bonds held during the fiscal year ended September 30, 1958.

(2) All bonds are in the name of The American Society of Tropical Medicine and Hygiene and are kept in a safety deposit box in the Coconut Grove Bank, Miami, Florida, where they were counted on October 13, 1958 in company with the Secretary-Treasurer, Dr. Hill.

(3) The interest earned on the Series J Bonds is available for current expenditures. However, no interest will be received until maturity date unless the bonds are redeemed prior to that time. The bonds have increased in redemption value each year. As of September 30, 1958, the total redemption value of the Series J bonds was $213.95.

(4) Interest on Series K Bonds is available for reviews in the *Journal* and for publishing abstracts of papers on the program. Interest available for this purpose is summarized below:

Total interest available as of September 30, 1957	$467.31
Interest collected during fiscal year	552.00
Total interest available	$1,019.31
Expenditures during current period	345.23
Balance of interest available as of September 30, 1958	$674.08

EXHIBIT B

The American Society of Tropical Medicine and Hygiene

Miami, Florida

COMPARATIVE STATEMENTS OF CASH RECEIPTS AND DISBURSEMENTS

For the Fiscal Years Ended September 30, 1958 and 1957

	Year ended September 30, 1958		*Year ended September 30, 1957*	
Beginning cash balances, September 30, 1957 and 1956		$3,359.64		$2,213.32
Receipts:				
Membership dues:				
1959	$96.00			
1958	8,625.50		$61.50	
1957	210.50		8,504.00	
Prior years	28.00	8,960.00	318.50	8,884.00
The American Journal of Tropical Medicine and Hygiene profit for calendar years 1957 and 1956		27.57		1,937.57
Profits from annual meetings at Philadelphia, Pennsylvania (1958) and New Orleans, Louisiana (1957)				
Share of income from exhibits	$339.97		$328.19	
Registrations	229.17		108.47	
Refunds on advances to local committees on arrangements		569.14	300.00	736.66
Interest earned on U. S. Savings Bonds (Exhibit A, Schedule 1)		552.00		552.00
Refund from Dr. Larsh for unused portion of expense advance New Orleans meeting				11.71
Refund from insurance agent covering unused portion of old Secretary-Treasurer's fidelity bond due to change in officers				23.13
Miscellaneous transactions in connection with dues and subscriptions		40.00		35.84
Reimbursement of unused portion of petty cash advanced to Dr. Larsh upon completion of term as Secretary-Treasurer				7.69
Publication of *Tropical Medicine and Hygiene News*		500.00		
Payment received on a life membership from E. N. MacDonald		100.00		
Use of stencils by Lea and Febiger		24.00		
Total cash available		$14,132.35		$14,401.92

Disbursements:				
Office expenses: (Exhibit B, Schedule 1)				
Editorial (Note 1)	$3,283.46		$3,318.26	
Secretary-Treasurer	1,912.44			
Total office expenses	$5,195.90		$3,318.26	
Williams and Wilkins for subscriptions to the *Journal*	6,678.00		6,738.00	
Publication of abstracts (Exhibit A, Schedule 1)	345.23	12,219.13	986.02	11,042.28
Ending cash balances September 30, 1958 and 1957		$1,913.22		$3,359.64
Ending cash balances, September 30, 1958 and 1957		$1,913.22		$3,359.64
Consisting of:				
Cash in bank		$1,913.22		$3,359.64

Note 1. The editorial expenses for the fiscal year ended September 30, 1957 were not reimbursed to Dr. Hackett until October, 1958. Of the figure $3283.46, $2010.70 represents editorial expenses of the fiscal year ending September 30, 1957 and $1272.76 represents editorial expenses of the fiscal year ending September 30, 1958. (For an itemized comparison of the editorial expenses for the two fiscal periods, see Exhibit B, Schedule 1).

EXHIBIT B

Schedule 1

The American Society of Tropical Medicine and Hygiene

Miami, Florida

OFFICE EXPENSES

For the Fiscal Years Ended September 30, 1958 and 1957

	Year ended September 30, 1958		*Year ended September 30, 1957*	
Expenses	*Editorial*	*Secretary-Treasurer*	*Editorial*	*Secretary-Treasurer*
Secretarial assistance	$769.54	$935.00	$773.00	$1,400.00
Honorarium, assistant to editor	75.00		750.00	
Travel and meetings	81.39	11.58	319.37	180.00
Postage	229.92	187.25	133.60	224.28
Printing		352.30	25.17	766.09
Office supplies	101.33	74.14	3.35	81.77
Telephone and telegraph	15.58	8.37	6.21	3.77
Auditing fee		175.00		200.00
Medals		22.38		
Freight and express		32.95		42.34
Corporate registration		30.00		30.00
Contribution to National Society of Medical Research		25.00		25.00
Insurance				156.25
Moving Secretary-Treasurer's equipment to Miami, Florida				164.14
Miscellaneous		58.47		44.62
Total office expenses	$1,272.76	$1,912.44	$2,010.70*	$3,318.26

* As of September 30, 1957 the editorial expenses for the fiscal year then ended had not been reimbursed to Dr. Hackett. These expenses were reimbursed in October, 1958. However, to provide a comparison between the two fiscal years, these expenses have been shown as though they were reimbursed by the organization in the fiscal year ended September 30, 1957.

EXHIBIT C

Tropical Medicine and Hygiene News

Bethesda, Maryland

COMPARATIVE BALANCE SHEETS

September 30, 1958 and 1957

Assets

	September 30, 1958	*September 30, 1957*
Current assets:		
Cash on hand and in bank (Exhibit D)	$2,193.91	$1,598.41
Accounts receivable	132.00	34.25
Stamps and postage deposit on metered mail	15.97	42.07
Inventory ("Health Hints for the Tropics")	287.62	154.00
Total Assets	$2,629.50	$1,828.73

Net Worth

Retained earnings:		
Balances September 30, 1957 and 1956	$1,828.73	$1,832.51
Increase for years ended September 30, 1958 and 1957	800.77	(3.78)
Total Net Worth (Exhibit A)	$2,629.50	$1,828.73

EXHIBIT D

Tropical Medicine and Hygiene News

Bethesda, Maryland

COMPARATIVE STATEMENTS OF CASH RECEIPTS AND DISBURSEMENTS

For the Fiscal Years Ended September 30, 1958 and 1957

	September 30, 1958		*September 30, 1957*	
Beginning cash balances, September 30, 1957 and 1956		$1,598.41		$1,438.99
Receipts:				
Advertising	$1,375.00		$1,875.00	
Sale of supplements	1,846.92		740.87	
American Foundation for Tropical Medicine	721.00	3,942.92	614.42	3,230.29
		$5,541.33		$4,669.28
Disbursements:				
Printing of *Tropical Medicine and Hygiene News* and Membership List (Note 1)	$888.00		$1,334.40	
Printing of "Health Hints for the Tropics"	851.34		281.91	
Copy preparation of *News* and list (Note 1)	547.00		813.50	
Postage and express	164.78		118.94	
Office supplies (Note 1)	66.37		244.02	
Secretarial help	141.47		125.29	
Deposits on postage meter account	100.00		140.00	
Travel expenses (editor)	86.53			
Transferred to American Society of Tropical Medicine and Hygiene	500.00			
Miscellaneous	1.93	3,347.42	12.81	3,070.87
Ending cash balances, September 30, 1958 and 1957		$2,193.91		$1,598.41

Consisting of:		
Cash in Bank of Bethesda	$1,632.24	$1,595.80
Undeposited checks (Deposited October 2, 1958)	559.27	
Cash on hand	2.40	2.61
Total Cash on hand and in bank (Exhibit C)	$2,193.91	$1,598.41

Note 1. The increased disbursements indicated in 1957 in these accounts were made in connection with the preparation of a new membership list during the fiscal period ended September 30, 1957.

As shown in the auditor's report above, our cash on hand has decreased some $1,400 during the year, to a little over $1,900.00. But as was also pointed out in last year's report, this amount does not reflect the true state of affairs. Against the amount of cash on hand, we have various appropriated items, as follows:

Journal Fund (extra pages for Journal)	$1,904.38
Interest on K bonds, part of which is to be used for publication of Transactions	674.08
Life Membership payment (for investment) (the second instalment was rec'd in Oct.)	100.00
Total	$2,678.46

The Treasurer was forced to use these funds, as he has for several years, for current expenses. Actually then, we have a deficit of $765.24 instead of a surplus.

In addition, it will be noted that (and this was due to our precarious financial situation and the necessity to keep a balance on hand) we have deferred payment to our efficient and devoted secretarial assistant for services rendered, amounting to $1,000 in 1957, and $1,465 in 1958, which adds $2,465 to our operating deficit, making a grand total of $3,230.24 in the red.

However, the picture is not entirely gloomy.

The receipt of $500.00 from the *News* toward the end of the fiscal year was a real boon; and if the estimated $1,311.68 profit from publication of the *Journal* materializes, these receipts, plus the expected increase in revenue from membership dues will go far toward alleviating, if not curing, our present distressing financial situation, and without dipping into our investment assets.

Another encouraging feature has been the decreased editorial expenses for the year, the present Editor having been able to reduce his expenses very materially. For the 9 months of his incumbency he has spent $532.57. Furthermore, early in the year the Council voted to suspend publication of abstracts of papers presented at the annual meeting, which will also help to improve our financial situation.

The Council also authorized the Treasurer to sell K bonds up to a limit of $2,500 to help defray current expenses. Although the necessary formalities were completed, no bonds were sold, since we were able to maintain our cash balance by the measures noted above.

At the 1957 annual meeting, the Council authorized the President to appoint a Committee to advise the Treasurer on reorienting and reorganizing our investment portfolio, with a view to securing an increased yield therefrom. The two members of this Committee held diametrically opposed views, and dissolved itself without coming to any agreement. The Council was then polled and here again opinion was divided, but a majority favored selling our Government bonds and investing the proceeds in both high-grade "industrial" bonds, and in one of the open-end Funds, such as Massachusetts Investors Trust. To date, no action has been taken by the Treasurer, partly because of the increased cost of good bonds with a consequent decrease in yield, (this having made government bonds relatively more attractive) and partly and principally because the Treasurer did not feel that he had sufficient competent and disinterested advice for him to proceed with safety and assurance.

During the year, a routine request to one of the Foundations for a subvention brought out the fact that the Society did not have a tax-exempt status, with all the consequences which could ensue from this oversight. This status was secured from the Internal Revenue Service, but we were then liable for withholding and FICA taxes for 1957 and succeeding years, which were paid up to date. The amount paid is included under secretarial assistance, and amounts to $135.00, with $45 still due for the last quarter of the fiscal year.

Recommendation. For the fiscal year 1958–1959 it is recommended that the Treasurer be authorized to expend a sum not to exceed $150 per month for secretarial assistance.

Respectfully submitted,
ROLLA B. HILL,
Treasurer

The Treasurer's report was accepted, with thanks to this Officer for his efficient work.

TABLE 1

The Journal after 11 years of operation

Items	1947*	1957*	1958
Papers published	83	127	98†
Contractual pages	—	900	600‡
Published pages	804	1,134	680§
Pages paid by contributors	398	294	137‡
Amounts paid by contributors		$4206.35	$3061.93†
Cost of printing per page	$9.81	$18.00	$21.78‡
Member subscriptions	877	1,124	1,128†
Nonmember subscriptions	1,215	1,296	1,207†
Total revenue	10,584	$20,280.37	$26,149.92‡
Production costs	6,863	$21,191.53	$23,526.56‡
Surplus (after dividing 50-50 with publishers)	3,721	$27.57¶	$1,311.68‡

* 6 issues (Vol. 6) = Calendar year.

† Actual, Vol. 7.

‡ Estimated.

§ Equivalent to 1186 old format.

¶ Deficit = $911.16; Income from back volume and reprint sales = $966.31 = surplus of $55.15 ÷ 2 = $27.57.

TABLE 2

Financial statement

	Vol. 5–6 (6 issues from Sept. 1956–July 1957)	Vol. 6–7 (6 issues from Sept. 1957–July 1958)
Income:		
Paid subscriptions	$19,245.82	$19,141.63
Space sales for advertising	1,731.18	1,845.13
Paid by contributors for extra pages	4,356.00	3,826.54
Net from reprint sales	553.99	594.62
Back-volume sales	400.00	742.00
Total income	26,286.99	26,149.92
Production cost:		
Printing of text	18,981.62	17,950.23
Printing advertisements	526.30	733.96
Distribution and other costs	1,190.00	1,112.37
Overhead	3,375.00	3,730.00
Total cost	24,072.92	23,526.56
Surplus (income less cost)	2,214.07	2,623.36
Amount due ASTMH (50%)	1,107.04	1,311.68
Circulation and size:		
Paid subscriptions (members)	1,101	1,127
Paid subscriptions (nonmembers)	1,267	1,207
Total paid subscriptions	2,368	2,334
Pages:		
Text	1,142	824*
Advertising	59	42
Total pages	1,201	866
Average cost of printing a page of text	$16.62	$21.78

* Mostly new, double-column, format.

TABLE 3

Classification of papers by subject

	Vol. 7, Nos. 1–6
Protozoology	18
Helminthology	25
Protozoa and Helminths	4
Virology	16
Bacteriology	8
Entomology	11
Malacology	5
Clinical	1
Nutrition	1
Mycology	1
Pharmacology	2
Miscellaneous	6
	98

Following discussion of the report, the Treasurer was instructed to pay the secretarial assistant the back salary now in arrears.

The recommendation in the report was approved.

The report of the Editor of the *American Journal of Tropical Medicine and Hygiene* from November 6, 1957, to November 5, 1958, was presented as follows:

After a new Editor was appointed in November, 1957, as successor to Dr. Lewis W. Hackett, it was mutually agreed that the next issue (January, 1958) would best be brought out under Dr. Hackett's continued direction since he had already brought it near to completion. Meanwhile the new Editor was arranging his schedule, enlisting the aid and support of an Editorial Board, and taking over partially completed manuscript work, proofreading, etc. Thus, though the names of the new Editorial Board and Editor appeared at the masthead of the January issue, it was really the work of Dr. Hackett and the previous Editorial Board. It was a real hybrid, especially as it was partly in broad format and partly in double-column format. It would have been too expensive to reset, in double column, articles already set in broad format.

The double-column format is an important improvement and it saves a considerable part of the costs of paper and presswork, but it does not save anything on two of the most expensive items in publishing: composition or typesetting, and illustrations. These get more expensive every year. As pointed out by Dr. Hackett in his Annual Report last year, since 1947 the surplus margin of the Journal has narrowed so that, in the calendar-year-end financial report of the printer in 1957, a surplus of some thousands dwindled to $27.57! And this in spite of the fact that Dr. Hackett had that year collected $4,206.38 from contributors (Table 1). At least we had the comfort of starting in 1958 not owing anyone anything. Better still due to Dr. Hackett's good husbandry, we also had a reserve of some unused pages of our contractual 900 annual pages (516 pages in double column).

Because of this surplus of unused pages, and because of payments of $3,061.93 in 1958 by contributors (i.e., by their sponsoring institutions, research grants, etc.) as of now we owe no one anything. Our estimated surplus for this year amounts to $1,311.68 (Table 2). Besides, we still have a reserve of some unused pages worth about $1,200.00 However, like some modern moneys, these unused pages dwindle in value because each day they cost more to print. These pages are cumulative from volume to volume.

With the aid of anticipated increased revenue from subscriptions, and possibly from other sources which we may be able to develop, we hope to continue to write our financial records with black (or at least blue) ink. Summarizing the financial situation we may say that, while at present the Journal must not be looked to as an important source of revenue for the Society, we are not in debt, have a modest reserve, and hope to improve our financial status in the future.

As for the content of the Journal, due to the fine cooperation of contributors and the expert, often laborious, and always time-consuming, assistance of the members of the Editorial Board, both past and present, as well as of numerous other experts not on the Board or even members of the Society, we feel that the scientific quality of the Journal has continued high. As shown in Table 3, the numbers of manuscripts in different fields have shown some minor fluctuations. With only the present year's experience to compare with previous data it is difficult to say what these changes may portend, if anything.

With regard to review papers, provided for by a previous action of the Council and Editorial Board, none has been received for publication. We are preparing for the printer a separate supplement on *Malaria in Saudi Arabia* by R. H. Daggy which it is hoped can be ready to mail with the March issue.

The register of manuscripts from Nov. 6, 1957, to Nov. 3, 1958, is approximately as follows:

Received	*Accepted*	*Rejected*	*Withdrawn*	*In process*
149	102	28	8	11

The final status of some manuscripts has not yet been determined.

The Journal anticipates taking appropriate further notice of the passing of a valued and honored colleague and a long-time member and former President of this Society, Asa C. Chandler.

Respectfully submitted,
MARTIN FROBISHER,
Editor

The report of the Editor was accepted as read, with appreciation and thanks for his efficient work as Editor. In discussion of the report, it was emphasized that while the amount of reading matter has remained at the same level, the number of pages and hence the thickness of each issue has decreased because of the double-column format.

The report of the Editor of TROPICAL MEDICINE AND HYGIENE NEWS, October 1, 1957, to September 30, 1958, was presented as follows:

Six 24-page issues of *Tropical Medicine and Hygiene News* were published on schedule. The *News* was sent to all members of the Society and to between 600 and 700 interested nonmembers, most of whom live outside the United States. There were no substantial changes in format or editorial policy. However, there was increased use of pictures and an effort was made to further increase the coverage of news from abroad. Although this effort was partially successful, it is hoped that there will be greater success in the future. Also, the Editor of the Society has decided, at least for a time, to reduce the number of book reviews publised in the Journal and to send most of the books submitted for review to the News. This change is welcomed. It should increase the value of the News and will probably provide a wider reading public for the reviews.

Reports from members of the Society and from readers outside the United States indicate that the News is widely read and is a very useful means for spreading information related to our field.

The income to the *News* account was derived from three principal sources, (1) revenue from advertising, (2) revenue from the sale of "Health Hints for the Tropics," and (3) payments from the American Foundation for Tropical Medicine for printing and mailing the *News* for their mailing list. Advertising revenue fell below a desirable level despite the continued and conscientious efforts of Mr. Quarterman. His work throughout the year is gratefully acknowledged. It is felt that some way must be found to increase income from advertising if the *News* is to continue as a sound venture. Due to unusual factors, revenue from the sale of "Health Hints for the Tropics" far exceeded all previous records (11,600 sold) and accounts for the rise in the net worth of the venture. Sales for 1958–1959 will probably be much smaller than those of the past year.

In August a secretary-clerk was employed part-time to take care of many of the details of *News* affairs. This will increase costs somewhat because some of this work was done previously by the family of the editor at no cost.

It is expected that increased postal rates, which will take effect gradually during the next year, will raise the cost of the *News* activity by about $200.00 per year. This and other factors predict that the coming year may be a lean one unless advertising revenue is greatly increased.

Our most consistent advertiser was Burroughs-Wellcome and Company which took a page in every issue. Winthrop Laboratories, United Fruit Company, Pfizer Laboratories, and Shell Chemical Corporation also chose to advertise in our pages.

The editor wishes to acknowledge with thanks the help and advice of the Editor of the Society and the Secretary-Treasurer. He solicits the criticisms and suggestions of the membership and urges all to call to his attention items worthy of publication.

Respectfully submitted,
LOUIS J. OLIVIER,
Editor

The report of the Editor of the *News* was accepted as read, with a vote of thanks and appreciation to the Editor for his excellent work.

REPORTS OF COMMITTEES

Walter Reed Medal. The Committee, after receiving nominations from several sources, voted that the Walter Reed Medal should be presented to Dr. Willard H. Wright at the 1958 meeting of the Society.

Charles Franklin Craig Lecture. The Committee, composed of Drs. Henderson, Whayne and McCoy, gave careful consideration to the selection of a suitable and timely speaker to address the Society on this twenty-third lecture of the series. In view of his various qualifications, and particularly his achievements and interest in the enteric viruses as a challenging public health problem in tropical medicine and hygiene, the Committee unanimously decided to invite Dr. Jerome T. Syverton to deliver this annual lecture. Dr. Syverton kindly accepted.

Honorary membership. The Committee agree that no nominations for Honorary membership should be submitted this year.

Membership. The membership Committee examined the credentials of 71 applicants for membership in the Society, and approved 70 of these.

The total membership on September 30, 1958 was 1,231, compared to 1,229 a year previously. There are 12 Honorary Members, 11 Emeritus Members, 2 Life Members and 1086 Active Members, plus an additional 120 members in arrears of dues for one or more years. Continuing accepted Society policy, the Committee has made no concerted attempt to solicit new members.

Nominating. This Nonimating Committee drew up a slate of candidates for the various offices, which was submitted by ballot to the membership.

Program. The Committee reviewed the abstracts of all papers submitted for presentation at the annual meeting, the majority of which were accepted. Due consideration was given to the 1957 Council directive that only single sessions should be held. The printed programs were mailed to all members early in September. In all, seventy applications for a place on the program were accepted.

Public Relations. This Committee has been relatively inactive this year, and has confined its activities again to supporting the allotment of budgets by the Congress for the Institute of Allergy and Infectious Diseases of the National Institutes of Health. At the request of Mr. David Crockett of the Massachusetts General Hospital, several letters were written to congressman in support of the above. It is gratifying that our efforts on behalf of the Institute have been successful.

Development and Needs. The National Research Council has finally succeeded in obtaining from both governmental and private sources the necessary funds to establish the Commission on Medical and Hygienic Problems of the Tropics, a project initially conceived and sponsored by the American Society of Tropical Medicine and Hygiene. Dr. Willard H. Wright has been appointed as full-time director of this Commission beginning January 1, 1959.

Our Society, as well as other interested groups, is looking to the proposed Commission to provide a foundation as well as a plan for the role to be played by the U. S. A. in future research on those problems in human and veterinary medicine, hygiene, nutrition and climatology that constitute the greatest impediment to the future development of the tropics for the benefit of its present inhabitants as well as for all mankind.

All of the above reports were accepted as presented, with thanks to the members of the various Committees for their efforts. The Council was particularly pleased that the National Research Council has secured funds for the establishment of a Commission on Medical and Hygienic Problems of the Tropics.

REPORTS OF REPRESENTATIVES TO ORGANIZATIONS

National Research Council, Division of Medical Sciences. Submitted by Leon Jacobs.

The Council met on March 30 to April 1, 1958. This was a full meeting of the entire Council, comprising all its divisions. Special attention at the plenary session was devoted to the following five topics: 1) Education in science, 2) International aspects of science, 3) Beyond the geophysical year, 4) Information and documentation in science, and 5) Problems in the stimulation of research. Of special interest to this Society is the prospect mentioned in the discussion of the utilization of International Geophysical Year facilities, that such facilities in remote areas might be made available for biological and medical research in the future. This could, possibly, represent an opportunity for study of tropical diseases at certain stations located at appropriate areas.

At the divisional meeting, the American Society of Tropical Medicine and Hygiene was elected to continued representation. This was the only encouraging note, from our standpoint.

American Foundation for Tropical Medicine. Submitted by Dr. E. L. Salisbury.

The scientific staff of the Liberian Institute in 1958 consisted of seven resident members, while two visiting scientists spent two and three months, respectively, at the Institute. It is anticipated that, in the future, the number of visiting scientists requesting facilities for work at the Institute will increase. A request has already been received from the Director of the Tropeninstitut, Hamburg, for permission to send a pathologist to the Institute for a 6-month period. W. H. O. also wishes to have one of their entomologists work at the Institute for a period of 1 year.

Dr. Robert S. Bray has been doing a good job as Acting Resident Director and is prepared to continue in this capacity until a resident director can be found.

OTHER OLD BUSINESS

Election of Officers and Councilors for 1958–59. The slate submitted by the Nominating Committee was put into ballot form and mailed to all voting members of the Society in March. The 222 votes received were tabulated by the Tellers appointed by the President, Dr. J. Walter Beck and Mrs. Mary W. Winter, with the following result:

President-elect . . . . . WILLARD W. WRIGHT
Vice-President . . . . . OLIVER R. MCCOY
Councilors . . . . . MARSHALL HERTIG
JOHN E. LARSH, JR.

It is to be regretted that more of the members do not return their ballots.

Leslie A. Stauber, who was appointed in 1957–1958 to fill out the unexpired term of Paul C. Beaver, who resigned when he was elected to the Vice-Presidency, was reappointed for an additional year, 1958–59, by the President, with the concurrence of the Council.

NEW BUSINESS

Committee appointments. The Council elected the following members to serve on the Nominating Committee for 1958–59: J. C. Swartzwelder, T. H. Weller, and J. M. Henderson.

The Council also appointed Clay G. Huff to serve on the Bailey K. Ashford Award Committee for a term of 6 years. The other members of this Committee at present are Martin D. Young and W. W. Frye.

Contribution to the National Society for Medical Research. The Council voted to again contribute $25.00 to this Society.

Ad hoc Committees for 1958–59. The Council authorized the President to appoint such Committees as he deemed necessary and advisable.

Amendment of the By-Laws of the Society.

1. At the 1957 annual meeting, the Council approved an increase in annual dues to $10.00. Consequently, the By-Laws, Article XI, first sentence, were amended to read as follows: "The annual dues of the Society shall be ten dollars which shall become due and payable on the first day of January of each year." The remainder of this Article remained unchanged.

2. It was announced that the membership had voted to abolish the "Fellows" class of membership. Therefore the Council voted to strike out Article X of the By-Laws which reads: "Fellows shall be elected by a two-thirds majority vote of the Officers and Councilors of the Society."

3. The Council agreed that in those cases where both husband and wife are active members of the Society, the latter, on request, may be absolved from payment of the second subscription to the Journal, but will still be liable for her general dues.

Age limit for the Bailey K. Ashford Award. During the year, the Eli Lilly Company, sponsor of the Bailey K. Ashford Award, proposed to the Chairman of this Committee that the age limit be raised to 40 years. This proposal was agreed to by the Council, which then voted to raise the age limit to 40 years.

New Membership List. The last Membership Directory was published in December, 1956. Since then, approximately 40% of the listings have changed. On motion, the Secretary was instructed to prepare a new Directory for publication as soon as possible, and was directed to charge a fee of $20.00 for each Directory supplied for commercial purposes.

Revision of "Health Hints for the Tropics." As shown in the report of the Editor of the *News*, Health Hints continues in active demand. It will soon be necessary to reprint this booklet, and a revision of the text is advisable. Dr. Olivier, who was responsible for the last revision, suggests that a special committee be put in charge of this task. On motion, it was agreed to revise "Health Hints," with the President empowered to appoint a Chairman of a Committee for this purpose, the Chairman to select those additional persons he deems necessary to serve with him on the Committee.

Request by the Basle Institut of Tropical Medicine to translate Health Hints. This Institute has requested permission to translate "Health Hints" primarily for the use of its students who may not read English. The Institute also inquired whether an arrangement for possible distribution of "Health Hints" in translation would be considered by the Society. On motion, it was agreed to give permission to the Institute of Tropical Medicine to translate "Health Hints" into French and German for distribution to their classes, and to offer the Institute the opportunity to negotiate a contract with this Society for the sale, in consultation with the Editor of the *News* and the Secretary of the Society, of these translations. The copyright of "Health Hints" is to remain the property of the Society.

1960 Meeting. The Council agreed to Los Angeles as the site of the 1960 meeting, which will be held jointly with the American Society of Parasitologists, and also agreed to the proposed date, November 2–5.

Joint meeting with ASP in 1963. The Council also agreed in principle to hold a joint meeting with the American Society of Parasitologists in Chicago in 1963.

Result of mail ballot on Constitutional Amendments. A proposed Constitutional Amendment having been received in accordance with regulations, and a vote having been approved by the Council, a mail ballot was circulated to all

voting members, and the results tabulated. The amendment (and consequent deletion) were passed by a vote of 196 to 35, which is more than the necessary two-thirds majority of votes cast.

The Constitution now reverts to its original (1952) form. Article II, Section 2, will now read "There shall be four classes of members: Active, Honorary, Life and Emeritus." Article II, Section 7, is deleted in its entirety. This section defines eligibility for election to Fellowship and specifies how Fellows shall be elected.

There being no further business, the meeting adjourned.

Report of the Resolutions Committee (George Bevier, Chairman)

1. It is requested that the Secretary of the Society be instructed to write and send resolutions of sympathy to the families of deceased members of the American Society of Tropical Medicine and Hygiene. During the current year the Society has lost Dr. Asa C. Chandler (President of the Society, 1955–56), Dr. Michael J. Takos, Dr. William W. Hersohn, Dr. Walter C. Humbert, Dr. Charles R. Rein, Dr. Edwin S. Gault, and Dr. Ralph F. Allen. Just prior to the meeting news came that it also lost Dr. George K. Strode.

2. To *Dr. Jerome T. Syverton* we extend the Society's sincere thanks and highest esteem for his presentation of the Twenty-third Annual Charles Franklin Craig Lecture, "*An Appraisal of Enterovirus Problems.*"

3. The Society expresses its appreciation to the organizations and individuals who have served as hosts for the Seventh Annual Meeting of the Society held in Miami Beach, Florida, November 5–8, 1958, or otherwise furnished helpful assistance:

A. The officers and members of the Society are deeply appreciative of the assistance and hospitality extended by the *University of Miami School of Medicine* and by members of its faculty and staff. THEREFORE BE IT RESOLVED that the thanks of the Society be expressed to the Dean, Dr. Homer F. Marsh and, through him, to the Faculty and Staff.

B. The assistance and cooperation of the staff of the *Deauville Hotel* contributed to the success of the meetings. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Society be expressed to Mr. E. J. Fried, Manager, and, through him, to members of the hotel staff.

C. *The Miami Beach Convention Bureau* supplied secretarial help and other assistance of great value. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Society be expressed to Mr. Thomas F. Smith, Director of the Bureau.

D. The Society appreciates the provision of a subvention by *Burroughs-Wellcome & Co.* THEREFORE BE IT RESOLVED that the thanks of the Society be extended to this company for its assistance.

E. The planning of the *Local Committee on Arrangements* was an essential contribution to the success of the meeting. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Society be expressed to Dr. Ralph Jones, Jr., and J. Walter Beck, co-Chairmen, and Committee members as follows: Drs. Harvey Blank, N. Joel Ehrenkranz, James S. Ferguson, M. Eugene Flipse, David S. Howell, Ulfar Jonsson, Fred. H. Stutz, Carlos M. Vilar-Alvarez and Mary W. Winter.

F. The hospitality sessions were a most important contribution to the pleasure of the individual members and guests and the success of the meeting as a whole. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Society be extended to *Rooley, Inc.*, and to *Parke, Davis & Co.*, for providing these sessions.

G. The commercial exhibits added materially to the interest of the meeting. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Society be expressed to *Mrs. Herma H. Hoefler* who served as Chairman of the Commercial Exhibits Arrangements and through her to the commercial exhibitors. She also had charge of the Scientific Exhibits.

H. The Scientific Exhibits were of outstanding interest. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Society be expressed to the individuals who contributed these exhibits, through their institutions as follows: the *Rockefeller Institute for Medical Research;* the *National Institutes of Health;* the *Louisiana State University School of Medicine;* the *Veterans Administration Hospital* and the *Charity Hospital of La., New Orleans;* the *State Colony and Training School, Pineville, La.; Hospital San Juan de Dios, San José, Costa Rica;* the *Institute for Scientific Research in Central Africa,* and the *Walter Reed Army Institute of Research.*

I. The interest and planning of the *Committee for Ladies Entertainment* contributed greatly to the pleasure of the ladies attending the meeting. THEREFORE BE IT RESOLVED that the Society express its thanks and appreciation to Mrs. Ralph Jones, Jr., Chairman and Mrs. Rolla B. Hill, Honorary Chairman, and through them to the other members of the Committee: Mrs. J. Walter Beck, Mrs. Harvey Blank, Mrs. William B. Deichmann, Mrs. Ulfar Jonsson, Mrs. Kenneth

Savard, Mrs. G. W. Runyan and Mrs. Franz Stewart.

4. *Editors of Society Publications.* Dr. Martin Frobisher, Editor-in-Chief of the *American Journal of Tropical Medicine and Hygiene*, and Dr. Louis J. Olivier, Editor of *Tropical Medicine and Hygiene News*, have maintained the high standard of these publications. THEREFORE BE IT RESOLVED that expressons of appreciaton of the Society be extended to these members.

5. *Visitors from abroad.* The Society welcomes expecially the visitors from abroad who attended this meeting: R. N. Chaudhuri, Director of the School of Tropical Medicine, Calcutta; Svasti Daengsvang of Bangkok; Marcil P. De Smet of the Belgian-American Education Foundation in the Belgian Congo; Reynold Dolly, Medical Director of Texaco, Trinidad, B. W. I.; Roberto Gomez, Medical Director of the Texas Oil Company of Colombia; Alfredo Landinez, Medical Director of the Colombian Petroleum Co.; and the South American Gulf Oil Co., Cúcuta, Colombia; Somsak Phansomboon of Bangkok; Stanley Bell, London School of Tropical Medicine; and Otto Kranendonk, Amsterdam, Holland.

SCIENTIFIC AND SPECIAL SESSIONS

Six scientific sessions were held, at which 64 papers were presented. The titles are listed below, but in accordance with a Council directive, the summaries are not given. In addition, seven motion pictures were shown, partly during the regular meetings, and partly in two special sessions. An Annual Dinner session was also held, at which Ralph Jones, Jr., served as Toastmaster. At the dinner, W. A. Sodeman presented the Walter Reed Medal to Willard H. Wright. Introduced by Thomas H. Weller, President Donald L. Augustine gave the Presidential Address, "The Worm Turned—Action and Reaction."

PAPERS

1. THE TWENTY - THIRD ANNUAL CHARLES FRANKLIN CRAIG LECTURE: *An Appraisal of Enterovirus Problems.* JEROME T. SYVERTON, University of Minnesota Medical School, Minneapolis, Minn.
2. *The Epidemiology of Kyasanur Forest Disease in India.* TELFORD H. WORK, Virus Research Centre, Poona, India.
3. *The Use of Correlation Maps in Tropical Research.* LOUIS VAN DEN BERGHE, Institute for Scientific Research in Central Africa, Belgian Congo.
4. *The Campaign against Yaws in Netherlands New Guinea.* OTTO J. M. KRANENDONK, World Health Organization, Netherlands, New Guinea.
5. *The Status of Malaria and* Aedes aegypti *eradication in the Caribbean, 1958.* P. F. DE CAIRES, Pan American Sanitary Bureau, Caracas, Venezuela.
6. *Epidemiologic Trends in Malaria in the United States since 1953.* FREDERICK L. DUNN AND JACOB C. BRODY, Communicable Disease Center, Atlanta, Ga.
7. *Electrophoretic Patterns of Sera from Mice Immunized with* Plasmodium berghei. NORMAN T. BRIGGS. BETTIE C. GARZA and EDITH D. BOX, University of Texas, Galveston, Texas.
8. *Thiamine in Rats with Infections of* Plasmodium berghei. IRA SINGER, Rockefeller Institute for Medical Research, New York, N. Y.
9. *Attempt to Eradicate Malaria with Pyrimethamine in Areas of Out-of-doors Transmission in Venezuela.* ARNOLDO GABALDON AND LACENIO GUERRERO, Division of Malariology, Maracay, Venezuela.
10. *Intramuscular Propoquin in Clinical Malaria.* MARK T. HOEKENGA, United Fruit Company, Puerto Armuelles, Panama.
11. *Recent Developments in Insecticide Application Equipment and their Relation to Economy in Malaria Eradication Programs.* L. B. HALL, R. P. LONERGAN AND K. D. QUARTERMAN, Communicable Disease Center, Savannah, Ga.
12. *A Note on Viruses Isolated from Argus Ticks in Egypt.* R. M. TAYLOR AND H. S. HURLBUT, Yale University, New Haven, Conn., and Naval Medical Research Institute, Bethesda, Md.
13. *Naturally Acquired Yellow Fever Immunity in Munzo, Colombia.* J. AUSTIN KERR, MANUEL ROCA-GARCIA AND JOHN C. BUGHER, The Rockefeller Foundation, New York, N. Y., and Lederle Laboratories, Pearl River, N. Y.
14. *Field Differentiation of Encephalitis Virus Infection versus Transmission Rates in Mosquito Vectors.* WILLIAM C. REEVES, R. EDWARD BELLAMY AND ROBERT P. SCRIVANI, University of California, Berkeley, Cal., and Communicable Disease Center, Bakersfield, Cal.
15. *Laboratory Studies on the Transmission of Japanese Encephalitis Virus by the Mosquito* Culex gelidus *Theobald.* D. J. GOULD, H. C. BARNETT AND W. SUYEMOTO, Walter Reed Army Institute of Research, Washington D. C.
16. *An Epidemic of St. Louis Encephalitis in the Rio Grande Valley, Texas, 1957, Involving an Immune and a Susceptible Population.* JACOB

A. Brody and Frederick L. Dunn, Communicable Disease Center, Atlanta, Ga.

17. *Effect of Gamma Radiation on some Insects Affecting Man.* M. M. Cole, G. C. LaBrecque and G. S. Burden, U. S. Department of Agriculture, Orlando, Fla.
18. *Interrelationship of Water and* Hermetia *Breeding to* Musca domestica *Production in Human Excrement.* J. W. Kilpatrick and H. F. Schoof, Communicable Disease Center, Savannah, Ga.
19. *Effect of Temperature upon Growth and Development of* Trypanosoma cruzi *in Tissue Culture.* Franklin A. Neva and Mary F. Malone, Harvard University, Boston, Mass.
20. *Diagnosis of* Trypanosoma cruzi *Infection by Fluorescent Antibody Procedures.* Earl H. Fife, Jr. and Louis H. Muschel, Walter Reed Army Institute of Research, Washington, D. C.
21. *The Effect of Thiamine Deficiency on Susceptibility to Infection with* Trypanosoma cruzi. Robert G. Yaeger and O. Neal Miller, Tulane University, New Orleans, La.
22. *The Course of a* Trypanosoma duttoni *Infection Following Graded Injury to the Lymphoid-Macrophage System of Mice.* Bernard N. Jaroslow, Argonne National Laboratory, Lemont, Ill.
23. *The Virulence and Drug Susceptibility of Certain Strains of Trypanosomes of the* bruceievansi *Group Maintained by Syringe Passage.* F. C. Goble, B. Ferrell and A. B. Stieglitz, Ciba Pharmaceutical Products, Inc., Summit, N. J.
24. *Comparative Results of Subcutaneous Inoculation of Germfree and Conventional Guinea Pigs with two Strains of* Trichomonas vaginalis. Walter L. Newton, Lucy V. Reardon and Anna M. De Leva, National Institutes of Health, Bethesda, Md.
25. *An Enriched, Egg-extract Medium for the Cultivation of* Entamoeba histolytica *trophozoites in Fecal Suspensions Kept for Four Days at 25°C.* N. N. Gleason, Morris Goldman and R. K. Carver, Communicable Disease Center, Atlanta, Ga.
26. *Serological Reactions in Experimental Animals Inoculated with Amebic Antigens.* J. C. Moan, Mobac Laboratories, Lansdowne, Pa.
27. *Treatment of* Entameba histolytica *Infections with Fumagillin, with Special Reference to the Leucocyte Count.* A. Fritsch and H. B. Shookoff, New York City Department of Health, New York, N. Y.
28. *Antiamebic Studies on the Antibiotic Paromomycin (Humatin).* Paul E. Thompson, Anita Bayles, Sheila Fleming Herbst, Bronislawa Olszewski and Jack E. Meisenhelder, Parke, Davis and Co., Detroit, Mich.
29. *Paromomycin (Humatin) in the Treatment of Intestinal Amebiasis.* C. H. Carter, Sunland Training Center, Gainesville, Fla.
30. *Periodontosis and Amebiasis.* Harry Seneca and John K. Karnig, Columbia University, New York, N. Y.
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32. *Clinical Observations during Hetrazan Treatment of Patients with* Loa loa Infection. H. B. Shookoff, New York City Department of Health, New York, N. Y.
33. *The Mongolian Gerbil as a Laboratory Host of* Litomosoides carinii. J. Allen Scott, Etta Mae MacDonald and John H. Cross, Jr., University of Texas, Galveston, Texas.
34. *Studies on Immunity to* Schistosoma mansoni. *II. The Circumoval Precipitin Reaction to* Schistosoma mansoni *in Mice Treated with Fuadin (Stibophen).* R. Rodriguez-Molina, Jose Oliver-Gonzalez and Amina Rivera de Sala, San Patricio V. A. Hospital and University of Puerto Rico, San Juan, P. R.
35. *Observations on Growth Requirements of* Schistosoma mansoni *in Azenic Culture.* A. W. Cheever and T. H. Weller, Harvard University, Boston, Mass.
36. Schistosomiasis mansoni *in Mice Maintained on a Pantothenate Deficient Diet.* William B. DeWitt, National Institutes of Health, Bethesda, Md.
37. *Nutritional Studies in Schistosomiasis* mansoni. *The Role of Protein Quality and Caloric Intake.* José F. Maldonado and Conrado F. Asenjo, University of Puerto Rico, San Juan, P. R.
38. *Distributions of Eggs of Geographic Strains of* Schistosoma japonicum *in Various Organs of Infected Mice.* S. Y. Li Hsu and H. F. Hsu, State University of Iowa, Iowa City, Ia.
39. *Observations on an Acid-fast Pathogen of Fresh Water Snails.* Edward H. Michelson, Harvard University, Boston, Mass.
40. *Staining* Toxoplasma gondii *with Fluorescein-labelled Antibody.* R. K. Carver and Morris Goldman, Communicable Disease Center, Atlanta, Ga.
41. *The Prevalence of Toxoplasmosis in Wild and Domesticated Animals of the Memphis Region.* Don E. Eyles, Colvin L. Gibson, Nell Coleman, C. S. Smith, J. R. Jumper and Frances E. Jones, National Institutes of Health, Memphis, Tenn.
42. *Characteristics of the Toxoplasma Hemagglutination Test Antigen.* Milford N. Lunde

AND LEON JACOBS, National Institutes of Health, Bethesda, Md.

43. *Characteristics of Orally Induced Infections of* Toxoplasma gondii *in Mice.* FRANCES E. JONES AND DON E. EYLES, National Institutes of Health, Memphis, Tenn.
44. *The Nutritional Status of Haitian Children.* D. B. JELLIFFE, Tulane University, New Orleans, La.
45. *The Diarrhea of Travelers. Studies in Mexico.* B. H. KEAN, Cornell University, New York, N. Y.
46. *Serological Studies in an Outbreak of* Shigella flexneri 6 *in Adults.* M. R. SOCHARD, R. B. LINDBERG AND V. M. YOUNG, Walter Reed Army Institute of Research, Washington, D. C.
47. *The Effects of Chemical and Physical Agents on Filariform Larvae of* Ancylostoma braziliense. HARVEY BLANK, MARY W. WINTER AND J. WALTER BECK, University of Miami, Miami, Florida.
48. *A Report on the Laboratory Evaluation of a New Anticestode.* JOHN E. LYNCH AND JOAN E. MARGISON, Pfizer Therapeutic Institute, Maywood, N. J.
49. *The Use of Dithiazanine in the Treatment of Helminthiasis in Mexican Farm Laborers.* EDWARD D. WAGNER, FRANK R. LEMON AND HOWARD S. BURNETT, College of Medical Evangelists, Loma Linda, Cal.
50. *The Treatment of Pinworm Infections in Humans (Enterobiasis) with Pyrvinium Chloride and Pyrvinium Pamoate.* J. WALTER BECK, DIEGO SAAVEDRA, GUNNARD J. ANTELL AND BELEN TEJEIRO, University of Miami, Coral Gables, Fla.
51. *Enterobiasis Treatment and Prevention in an Institution.* Charles H. Carter, Sunland Training Center, Gainesville, Fla.
52. *A New Drug Active Against Human Hookworm.* MARTIN D. YOUNG, GEOFFREY M. JEFFREY, JOE E. FREED AND WILLIAM G. MOREHOUSE, National Institutes of Health, Columbia, S. C., and South Carolina State Hospital, Columbia, S. C.
53. *Use of Methoxsalen in the American Tropics.* MARK T. HOEKENGA AND HAROLD A. TUCKER, United Fruit Company, Puerto Armuelles, Panama, and The Upjohn Company, Kalamazoo, Mich.
54. *Investigation of Parasitic Infection in the Central Area of Philadelphia.* DANIEL WEINER AND MARION M. BROOKE, Communicable Disease Center, Atlanta, Ga.
55. *Balantidium Infections in Puerto Rican Patients.* K. G. DWORK AND H. B. SHOOKOFF, New York City Department of Health, New York, N. Y.
56. *Studies on* Echinococcus granulosus *in Mississippi. I. Epidemiology.* THOMAS J. BROOKS, JR. AND WILLIAM F. HUTCHISON, University of Mississippi, Jackson, Miss.
57. *A Three-year Epidemiological Study of Intestinal Parasites in a Group of Mental Patients.* GEOFFREY M. JEFFREY, National Institutes of Health, Columbia, S. C.
58. *The Use of an Indirect Hemagglutination Test for the Detection of Infection with* Clonorchis sinensis. GUILLERMO PACHECO, DALE E. WYKOFF AND RODNEY C. JUNG, Tulane University, New Orleans, La.
59. *Some Characteristics of the El Tor Vibrios Isolated from the 1957 Epidemic in Sulavesi Java.* S. T. W. TANAMAL, Y. WATANABE AND O. FELSENFELD, Public Health Laboratory, Macassar, Sulavesi, Indonesia, 406th Medical General Laboratory, U. S. Army, and Walter Reed Army Institute of Research, Washington, D. C.
60. *The Immunologic Basis of Host Specificity of the Sparganum Larva of* Spirometra mansonoides. JUSTUS F. MUELLER, State University of New York, Syracuse, N. Y.
61. *Physicochemical and Serological Studies of Antigens from* Trichinella spiralis *Larvae.* H. KENNETH SLEEMAN AND EARL H. FIFE, Walter Reed Army Institute of Research, Washington, D. C.
62. *Studies by Paper Electrophoresis of Sera from Man and Experimental Animals Infected with Various Helminths.* CHAUNCEY G. GOODCHILD AND IRVING G. KAGAN, Emory University, and Communicable Disease Center, Atlanta, Ga.
63. *An Evaluation of the Hemagglutination and Flocculation Tests in the Diagnosis of* Echinococcus *Disease.* IRVING G. KAGAN, DOROTHY S. ALLAIN AND LOIS NORMAN, Communicable Disease Center, Atlanta, Ga.
64. *Serology of Visceral Larva Migrans: In Vitro Precipitate Test.* L. J. OLSON, University of Texas, Galveston, Texas.

MOTION PICTURES

1. *Embryogeny of* Ascaris suum *in Time-Lapse, Phase-contrast Photography.* BENEDICT J. JASKOSKI, Loyola University, Chicago, Ill.
2. *Development of the Exoerythocytic Stages of Avian Malaria in Tissue Culture. Time-lapse Photography.* CLAY G. HUFF, ALLAN C. PIPKIN, A. BURNS WEATHERSBY AND DINNIEMAUD V. JENSEN, Naval Medical Research Institute, Bethesda, Md.
3. *Mystery of the Sea.* BRUCE M. HALSTEAD, College of Medical Evangelists, Loma Linda, Cal. Sponsored by the Rolley Company, Los Angeles, Cal.

4. *The Story of Kyasanur Forest Disease.* TELFORD H. WORK, Virus Research Centre, Poona, India.
5. *Life Histories of the Two Species of Echinococcus of Man.* THOMAS B. MAGATH AND JOHN H. THOMPSON, JR., Mayo Clinic, Rochester, Minn.
6. *Clinical Aspects of Kwashiorkor.* LOUIS VAN DEN BERGHE, Institute for Scientific Research in Central Africa, Belgian Congo.
7. *Control of Yaws and other Diseases in New Guinea.* OTTO J. M. KRANENDONK, World Health Organization, Netherlands New Guinea.

SCIENTIFIC EXHIBITS

1. *Phagotrophy in Malarial Parasites.* Electron micrographs. MARIA A. RUDZINSKA AND WILLIAM TRAGER, The Rockefeller Institute for Medical Research, New York, N. Y.
2. *Pre-erythrocytic Stages of* Plasmodium cynomolgi *and Other Primate Malarias.* Slides and micrographs. DON E. EYLES, MARTIN D. YOUNG G. M. JEFFREY AND CLINTON S. SMITH, National Institutes of Health, Memphis, Tenn. and Columbia, S. C.
3. *Effective Broad Spectrum Intestinal Anthelmintic Activity of Dithiazanine.* Models, graphs and color slides. J. C. SWARTZWELDER, W. W. FRYE, J. P. MUHLEISEN, J. H. MILLER, R. LAMPERT, A. PEÑA CHAVARRIA, S. H. ABADIE AND ASSOCIATES. Louisiana State University, Veterans Administration Hospital and Charity Hospital of Louisiana, New Orleans, State Colony and Training School, Pineville, La., and Hospital de San Juan de Dios, San José, Costa Rica.
4. *Use of Correlation Maps in Tropical Research.* Maps and Photographs. LOUIS VAN DEN BERGHE Institute for Scientific Research in Central Africa, Belgian Congo.
5. *Disease - Free Laboratory Animals.* M. B. STARNES, Walter Reed Army Institute of Research, Washington, D. C.
6. *A Year-long Survey of Creeping Eruption.* FLORIDA STATE BOARD OF HEALTH AND COMMUNICABLE DISEASE CENTER, Jacksonville, Fla., and Atlanta, Ga.

TRANSACTIONS OF THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

Seventh Annual Meeting

Miami Beach, Fla., November 5–8, 1958

Addendum

Winthrop Products Inc. has donated $500.00 to the Society to help defray the cost of the November 1958 meeting of the Society. The Resolutions Committee has passed the following Resolution:

Be it resolved that the thanks and appreciation of the Society be extended to Dr. James Pi-Sunyer, Medical Director, and through him to Winthrop Products Inc. for their contribution toward the expenses incurred in connection with the 1958 annual meeting.

ROLLA B. HILL
Secretary-Treasurer

NOTICE TO CONTRIBUTORS

The Journal welcomes papers which contribute to the purpose of the American Society of Tropical Medicine and Hygiene. This is the advancement of knowledge of tropical and subtropical medicine and hygiene through investigation, prevention, treatment and control of diseases and deficiencies. It draws upon medicine, biology, public health, nursing, engineering and allied fields of research and application.

The American Journal of Tropical Medicine and Hygiene combines the American Journal of Tropical Medicine and the Journal of the National Malaria Society and therefore has a special concern with the investigation, therapy and control of malaria in all parts of the world, as well as a broad interest in general parasitology and arthropod-borne diseases.

Papers are ordinarily published in the order of reception, which will entail a certain delay in their appearance because of the contractual limitation on the number of journal pages available in any year. Accepted articles which raise no question of scientific priority but for which immediate publication seems important will be inserted in the next available issue of the journal if the author or sponsoring institution will pay the cost, estimate of which may be obtained from the editor. The insertion of such articles does not affect publication of other manuscripts awaiting their turn, since the inserted articles constitute additional pages in the volume without additional cost to the subscriber.

Publication will be facilitated if the following suggestions are followed:

1. *Manuscripts* should be typewritten in *English* using *double spacing* on *one side* only of 8½ x 11 inch paper and submitted *in duplicate*. Double-spacing should be used throughout the manuscript including the list of references, footnotes, and quotations.

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